รายงานวิจัยฉบับสมบูรณ์

โครงการ: วิธีแก๊สโครมาโทกราฟ-แมสสเปกโตรเมทรีแบบใหม่สำหรับการวิเคราะห์ไอโอดีนในปัสสาวะและในบะหมี่กึ่งสำเร็จรูป

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ผู้วิจัย

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษา และสำนักงานกองทุนสนับสนุนการวิจัย

CONTENTS

วิธีแก๊สโครมาโทกราฟ-แมสสเปกโตรเมทรีแบบใหม่ สำหรับการวิเคราะห์ไอโอดีนในปัสสาวะและในบะหมี่กึ่งสำเร็จรูป

บทคัดย่อ

ไอโอดีนเป็นธาตุอาหารที่สำคัญต่อการเจริญเติบโตและการพัฒนาสมอง การขาดสารไอโอดีนจะทำให้การผลิตฮอร์โมนไทรอยด์บกพร่อง ดังนั้นจึงได้มีการเสริมไอโอดีนลงในอาหารชนิดต่างๆ เช่น เกลือเสริมไอโอดีน น้ำดื่มเสริมไอโอดีน และยาเม็ดแคปซูลเสริมไอโอดีน รวมถึงในซองเครื่องปรุงในบะหมี่กึ่งสำเร็จรูป ในการประเมินภาวะการขาดสารไอโอดีนนั้น นิยมติดตามระดับปริมาณไอโอดีนในปัสสาวะ ซึ่งไอโอดีนจะอยู่ในรูปของไอโอไดด์ไอออน ดังนั้นจึงจำเป็นที่จะต้องมีวิธีการวิเคราะห์ที่มีความไว ความถูกต้อง และความแม่นยำสูงสำหรับการวิเคราะห์ปริมาณไอโอดีนในปัสสาวะและอาหารเสริม ซึ่งจะมีระดับปริมาณไอโอดีนที่แตกต่างกันและมีแมทริกซ์หลากหลายชนิด ในงานนี้ วิธีแก๊สโครมาโทกราฟ-แมสสเปกโตรเมทรีได้ถูกพัฒนาขึ้นสำหรับการหาปริมาณไอโอไดด์ในระดับต่ำ โดยอาศัยการเกิดปฏิกิริยาออกซิเดชันของไอโอไดด์เป็นไอโอดีนด้วย 2-iodosobenzoate จากนั้นไอโอดีนที่เกิดขึ้นจะทำปฏิกิริยากับ *N*,*N*-dimethylaniline ในสารละลายฟอสเฟตบัฟเฟอร์ แล้วอนุพันธ์ที่เกิดขึ้นจะถูกสกัดด้วยวิธี solid-phase extraction และการสกัดแบบของเหลว-ของเหลว ตามด้วยการแยกและตรวจวัดด้วยเทคนิคแก๊สโครมาโทกราฟ-แมสสเปกโตรเมทรีต่อไป ภายใต้สภาวะที่เหมาะสม การประเมินประสิทธิภาพวิธีที่พัฒนาขึ้น พบว่า วิธีนี้มีมีความเป็นเส้นตรงที่ดี ความถูกต้องและแม่นยำสูง กราฟมาตรฐาน (10 – 250 ไมโครกรัม ไอโอไดด์/กิโลกรัม) ได้จากการพล็อตระหว่างอัตราส่วนของพื้นที่ใต้พีกของสารอนุพันธ์ต่อสารมาตรฐานอ้างอิงภายใน (diphenylamine) กับความเข้มข้นของไอโอไดด์ สัมประสิทธิ์ความเป็นเส้นตรงมากกว่า 0.998 ความเข้มข้นต่ำสุดที่วิเคราะห์ได้ คือ 3 ไมโครกรัม ไอโอไดด์/กิโลกรัม วิธีนี้ได้ถูกนำไปประยุกต์ใช้ในการวิเคราะห์ปริมาณไอโอดีนในปาสวะและเครื่องปรุงของบะหมี่กึ่งสำเร็จรูป พบว่า วิธีนี้ไม่สามารถใช้หาปริมาณไอโอดีนในปัสสาวะได้อย่างถูกต้อง เนื่องจากมีตัวรบกวนที่แตกต่างกันมากมายในแต่ละตัวอย่าง อย่างไรก็ตามวิธีนี้สามารถใช้หาปริมาณไอโอไดด์ในเครื่องปรุงของบะหมี่สำเร็จรูปได้อย่างมีประสิทธิภาพ โดยมีร้อยละการกลับคืนในช่วง 97% – 101%

คำสำคัญ: แก๊สโครมาโทกราฟ-แมสสเปกโตรเมทรี ไอโอดีน ไอโอไดด์ ปัสสาวะ บะหมี่กึ่งสำเร็จรูป

A new gas chromatography-mass spectrometry (GC-MS) method for the analyses of urinary iodine and instant noodle

Abstract

Iodine (I_2) is an essential element that plays an important role in growth and brain development. Failure to have adequate iodine leads to insufficient production of thyroid hormone. A control program of supplementary iodine in food such as iodized salts, iodized water and iodized oil capsules including iodized seasoning powder for instant noodle in being promoted and implemented. Urinary iodine excretion is currently the most reliable marker of iodine deficiency disorder. Iodine generally presents in urine as iodide ion (I^-). Therefore it is essential to have sensitive, accurate and precise method available to determine iodine in urine and supplementary food, which contains different levels of the element in the presence of various unanticipated matrix. In this work, a method of gas chromatography-mass spectrometry (GC-MS) has been developed for trace level determination of iodide based on the oxidation of iodide to iodine by 2-iodosobenzoate. The generated iodine then reacts with *N*,*N*-dimethylaniline in a phosphate buffer, following by extraction the product with solid-phase extraction and then liquid-liquid extraction for further separation and detection by GC-MS. Under the optimized condition, a satisfactory validation of data was achieved for linearity accuracy and precision. A calibration curve (10 – 250 μg I/kg) was obtained from a plot between the area ratio of the derivative to the internal standard, diphenylamine and the iodide concentration. The regression coefficient was higher than 0.998. The limit of detection was found to be 3 μg I/kg. This method was applied to determine iodide content in urine and seasoning powder for instant noodle. Unfortunately, the developed GC-MS method cannot accurately analyze for urinary iodine excretion because of the undesirable interference of unanticipated matrix. However, this method can be effectively applied to determine iodide fortified in the seasoning powder of instant noodle. Recoveries were ranged from 97% to 101%.

Keywords: Gas chromatography-mass spectrometry Iodine Iodide Urine Instant noodle

บทสรุปผู้บริหาร

(Executive Summary)

ปัจจุบันโรคขาดสารไอโอดีนยังคงเป็นปัญหาสาธารณสุขที่สำคัญของประเทศ หากขาดสารไอโอดีนจะทำให้เกิดภาวะผิดปกติต่อการเจริญเติบโตทั้งทางร่างกาย สมองและสติปัญญาของคนในทุกช่วงอายุ ถึงแม้ว่ากระทรวงสาธารณสุขจะมีมาตรการควบคุมและป้องกันภาวะการขาดสารไอโอดีน โดยการส่งเสริมให้ทุกครัวเรือนใช้เกลือและอาหารเสริมไอโอดีนแล้วก็ตาม แต่ก็ยังไม่ครอบคลุมประชากรส่วนใหญ่ของประเทศ ผู้ป่วยที่เป็นโรคขาดสารไอโอดีนไม่เพียงแต่จะพบมากในพื้นที่ชนบทห่างไกลทะเลหรือพื้นที่ราบสูง แต่ขณะนี้พบว่าผู้ที่อาศัยอยู่ในส่วนอื่นๆ ของประเทศหรือในประเทศที่เจริญแล้วทางเศรษฐกิจ ก็มีสิทธิ์เป็นโรคขาดสารไอโอดีนได้เช่นกัน โดยวิธีการที่นิยมใช้เพื่อแก้ไขปัญหาดังกล่าวคือ การเสริมไอโอดีนลงในอาหารชนิดต่างๆ เช่น ไข่เสริมไอโอดีน เกลือเสริมไอโอดีน ยาเม็ดแคปซูลเสริมไอโอดีน รวมไปถึงขนมขบเคี้ยวและเครื่องปรุงของบะหมี่กึ่งสำเร็จรูป

ในการประเมินผู้ป่วยว่าอยู่ในภาวะขาดสารไอโอดีนหรือไม่นั้น สามารถทำได้โดยการตรวจวัดปริมาณไอโอดีนที่ขับออกทางปัสสาวะ (iodine excretion) โดยองค์การอนามัยโลก ได้จำแนกระดับภาวะการขาดไอโอดีน ตามระดับความเข้มข้นของไอโอดีนในปัสสาวะ ดังตารางที่ 1

ตารางที่ 1 ระดับไอโอดีนในปัสสาวะมนุษย์ซึ่งบ่งบอกว่ามีสภาวะเสี่ยงต่อการขาดสารไอโอดีนหรือไม่

Median Urinary Iodine Concentration (μg/L)	**Iodine Nutrition**
< 20	Severe deficiency
20 - 49	Moderate deficiency
50 - 99	Mild deficiency
100 - 199	Optimal
200 - 299	More than adequate
> 299	Possible excess

ในปี 2550 มีรายงานว่า โรคขาดสารไอโอดีนในประเทศไทย กำลังมีแนวโน้มเป็นปัญหามากขึ้น จากการตรวจวัดไอโอดีนในปัสสาวะของหญิงมีครรภ์ที่มาคลอดในโรงพยาบาลต่างๆทั่วประเทศ ล่าสุดพบว่าสัดส่วนระดับไอโอดีนในปัสสาวะของหญิงมีครรภ์มีค่าต่ำกว่า 50 μg/L โดยค่าเฉลี่ยการขาดสารไอโอดีนทั่วประเทศสูงถึง 33.1% สูงกว่าเกณฑ์มาตรฐานที่องค์การอนามัยโลกกำหนด ส่งผลต่อการเกิดภาวะปัญญาอ่อน ปัญญาทึบ ไอคิวต่ำของเด็ก โดยพบว่าเด็กที่อาศัยอยู่บริเวณที่มีการขาดสารไอโอดีน มีสติปัญญาต่ำกว่าเด็กที่อยู่ในบริเวณที่ได้รับสารไอโอดีนเพียงพอถึง 13.5 จุดไอคิว

โครงการนี้ จึงสนใจพัฒนาวิธีวิเคราะห์ใหม่ที่สามารถใช้หาปริมาณไอโอดีน เพื่อประเมินสภาวะการขาดสารไอโอดีนของประชากรโดยการตรวจปริมาณไอโอดีนในน้ำปัสสาวะ ซึ่งเป็นดัชนีที่ใช้บอกสภาวะการขาดสารไอโอดีนในคนได้โดยตรง และยังจะได้นำวิธีที่พัฒนาขึ้นนี้ไปประยุกต์ใช้ในการหาปริมาณไอโอดีนในเครื่องปรุงของบะหมี่กึ่งสำเร็จรูปอีกด้วย ซึ่งจำเป็นต้องมีวิธีที่มีความไว (sensitivity) ความจำเพาะ (selectivity) และแม่นยำ (precision) สูง เนื่องจากปริมาณไอโอดีนในปัสสาวะของผู้ป่วยที่ขาดสารไอโอดีนอยู่ในช่วงที่ต่ำมาก คือ 20 – 99 μg/L นอกจากนี้ แมทริกซ์ต่างๆ ในปัสสาวะของแต่ละคน หรือแมทริกซ์ต่างๆ ในเครื่องปรุงแต่ละรสชาติของบะหมี่กึ่งสำเร็จรูปจะมีความซับซ้อนและหลากหลายมาก ดังนั้นวิธีที่จะใช้ตรวจวัดจึงต้องมีความจำเพาะสูง โครงการนี้จึงมุ่งที่จะพัฒนาวิธีวิเคราะห์ใหม่ที่อาศัยหลักการของเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโตรเมทรี เพื่อใช้ในการวิเคราะห์ไอโอดีนที่อยู่ในรูปของไอโอไดด์ โดยอาศัยปฏิกิริยาที่จำเพาะ คือ การใช้ปฏิกิริยาออกซิเดชั่นของไอโอไดด์ให้เปลี่ยนเป็นไอโอดีนด้วย 2-ไอโอโดโซเบนโซเอต (2-iodosobenzoate) ที่อยู่ในสารละลายฟอสเฟตบัฟเฟอร์ ไอโอดีนที่เกิดขึ้นจะทำปฏิกิริยากับสารอนุพันธ์ (derivatizing reagent) จากนั้นอนุพันธ์ของไอโอไดด์จะถูกสกัดไปอยู่ในชั้นของตัวทำละลายอินทรีย์ ตามด้วยการวิเคราะห์ด้วยเครื่องแก๊สโครมาโทกราฟีที่มีการตรวจวัดแบบแมสสเปกโตรเมทรี ซึ่งมีความจำเพาะ แม่นยำ และความไวสูง อีกทั้งการใช้ MS ยังเป็นการยืนยันว่าทำการวัดไอโอดีนเท่านั้น

ในงานวิจัยนี้ ได้ทำการศึกษาปฏิกิริยาการเตรียมอนุพันธ์ของไอโอไดด์ที่เหมาะสม วิธีการเตรียมตัวอย่างที่เหมาะสมเพื่อกำจัดตัวรบกวน และสภาวะในการแยกและตรวจวัดที่เหมาะสมของวิธีแก๊สโครมาโทกราฟี-แมสสเปกโตรเมทรี

ในการศึกษาปฏิกิริยาการเตรียมอนุพันธ์ของไอโอไดด์ที่เหมาะสม จะอาศัยการเกิดปฏิกิริยาออกซิเดชันของไอโอไดด์เป็นไอโอดีนในสารละลายฟอสเฟตบัฟเฟอร์ โดยไอโอไดด์ที่เกิดขึ้นจะถูกนำไปเตรียมอนุพันธ์ต่อไป จึงได้ทำการศึกษาหาสารก่อนอนุพันธ์ที่เหมาะสม 4 ชนิด คือ 1) 2,6-dimethylphenol 2) *N,N*-dimethylaniline 3) 2,6-dimethylaniline และ 4) 2,6-diisopropylaniline ซึ่งจะได้ผลิตภัณฑ์หรืออนุพันธ์ 4-Iodo-2,6-dimethylphenol, 4-iodo-*N,N*-dimethylaniline, 4-iodo-2,6-dimethylaniline และ 4-iodo-2,6-diisopropylaniline ตามลำดับ ผลการทดลองพบว่า *N,N*-dimethylaniline เป็นสารก่อนอนุพันธ์ที่เหมาะสมที่สุด โดยพิจารณาจากระยะเวลาในการเตรียมอนุพันธ์ และประสิทธิภาพของการแยกและตรวจวัดด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโตรเมทรี นอกจากนี้ ยังได้ทำการศึกษาหาสารมาตรฐานภายในที่เหมาะสม เพื่อเพิ่มประสิทธิภาพวิธีให้มีความแม่นและเที่ยงสูงขึ้น โดยได้ทำการศึกษาสารมาตรฐานอ้างอิงภายใน คือ 2,4,6-trichlorophenol และ Diphenylamine พบว่า Diphenylamine มีความเหมาะสมมากที่สุด โดยไม่รบกวนการวิเคราะห์และสามารถแยกและตรวจวัดด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโตรเมทรีได้เป็นอย่างดี

สำหรับการหาสภาวะที่เหมาะสมสำหรับการแยกและการตรวจด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโตรเมทรี ได้ทำการศึกษาพารามิเตอร์ต่างๆ เช่น อุณหภูมิของตู้อบ ส่วนฉีดสาร ส่วนตรวจวัด เทคนิคการฉีดสาร (Split/Splitless) ปริมาตรสารตัวอย่าง ช่วงการวัดมวลและวิธีการเก็บและแยกมวล (scanning mode) เป็นต้น ภายใต้สภาวะที่เหมาะสม อนุพันธ์และสารมาตรฐานอ้างอิงภายในสามารถแยกและตรวจวัดออกจากตัวรบกวนอื่นๆ ได้เป็นอย่างดี โดยในการประเมินประสิทธิภาพวิธี พบว่า วิธีนี้มีมีความเป็นเส้นตรงที่ดี ความถูกต้องและแม่นยำสูง กราฟมาตรฐาน (10 – 250 ไมโครกรัม ไอโอไดด์/กิโลกรัม) ได้จากการพล็อตระหว่างอัตราส่วนของพื้นที่ใต้พีกของสารอนุพันธ์ต่อสารมาตรฐานอ้างอิงภายใน (diphenylamine) กับความเข้มข้นของไอโอไดด์ สัมประสิทธิ์ความเป็นเส้นตรงมากกว่า 0.998 ความเข้มข้นต่ำสุดที่วิเคราะห์ได้ คือ 3 ไมโครกรัม ไอโอไดด์/กิโลกรัม

จากนั้นจึงได้นำวิธีที่พัฒนาขึ้นนี้ไปประยุกต์ใช้การหาระดับปริมาณไอโอดีนในปัสสาวะและปริมาณไอโอไดด์ในเครื่องปรุงของบะหมี่สำเร็จรูป แต่เนื่องจากแมทริกซ์ต่างๆ ในปัสสาวะของแต่ละคน หรือแมทริกซ์ต่างๆ ในเครื่องปรุงแต่ละรสชาติของบะหมี่กึ่งสำเร็จรูปจะมีความซับซ้อนและหลากหลายมาก ดังนั้นจึงจำเป็นที่จะต้องพัฒนาวิธีการเตรียมตัวอย่างที่เหมาะสม

เพื่อกำจัดตัวรบกวน จึงได้ทำการศึกษาวิธีการสกัดแบบต่างๆ เช่น การสกัดแบบของเหลว-ของเหลว การสกัดแบบ Solid-phase ทั้งแบบเดี่ยวและใช้ร่วมกันหลายชนิด เช่น แบบ reversed-phase แบบ ion exchange และแบบผสม นอกจากนี้ ยังได้ทำการศึกษาการสกัดแบบ Solid-phase ร่วมกับการสกัดแบบของเหลว-ของเหลว อีกด้วย พบว่า การเตรียมตัวอย่างทุกวิธียังไม่สามารถกำจัดตัวรบกวนได้หมด โดยคาดว่าไอโอดีนที่ได้จากการเกิดปฏิกิริยาออกซิเดชันของไอโอไดด์นั้น จะทำปฏิกิริยากับตัวรบกวนก่อน จึงทำให้ไอโอดีนเกิดปฏิกิริยาการเตรียมอนุพันธ์ได้ไม่ดี ทำให้ค่าร้อยละการกลับคืนต่ำกว่า 50 และแตกต่างกันในแต่ละชนิดตัวอย่างปัสสาวะอีกด้วย จึงไม่สามารถนำวิธีที่พัฒนาขึ้นนี้ ไปใช้ในการหาปริมาณไอโอดีนในปัสสาวะได้

อย่างไรก็ตาม เมื่อนำวิธีที่พัฒนาขึ้นนี้ ไปประยุกต์ใช้กับการวิเคราะห์ไอโอไดด์ในเครื่องปรุงรสชาติต่างๆ ของบะหมี่กึ่งสำเร็จรูปที่วางจำหน่ายในท้องตลาดเปรียบเทียบกับวิธี Inductively Couple Plasma – Mass Spectrometry (ICP-MS) พบว่า วิธีทั้งสองให้ผลการทดลองที่ไม่แตกต่างกันอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นที่ 95% เมื่อทำการเปรียบเทียบด้วยวิธีทางสถิติ paired-*t*-test

CHAPTER I
INTRODUCTION

Iodine is an essential dietary element for human. It is required for the synthesis of the thyroid hormones throxine T4 (3,5,3',5'-tetraiodothyronine), the active form, triiodothyronine T3 (3,5,3'-triiodothyronine) and the precursor iodotyrosines are also. Iodine intake is mainly as the inorganic iodide ion which is readily and completely absorbed form the gastrointestinal tract. Other forms of iodine are reduced to iodide before absorption [1]. Approximately 30% of absorbed iodide is used for hormonal synthesis. The Recommended Dietary Allowance (RDA) for iodine is the human iodine intake per day at each life stage. The recommended amounts were calculated from the measurement of iodine accumulation in the thyroid glands of individuals with normal thyroid function [2]. Table 1.1 shows RDA values for various stages of human life.

Table 1.1 Recommended Dietary Allowances (RDA) for Iodine

Recommended Dietary Allowance (RDA) for Iodine			
Life Stage	**Age**	**Male (μg/day)**	**Female (μg/day)**
Infants	0-6 months	110(AI)	110(AI)
Infants	7-12 months	130(AI)	130(AI)
Children	1-3 years	90	90
Children	4-8 years	90	90
Children	9-13 years	120	120
Adolescents	14-18 years	150	150
Adults	19 years and older	150	150
Pregnancy	all ages	-	220
Breastfeeding	all ages	-	290

Iodine Deficiency Disorders (IDD) is a world wide problem that affects the quality of life at all stages of growth and development. The effects of iodine deficiency in various developmental stages are shown in Table 1.2.

Table 1.2 The effect of iodine deficiency for various developmental stages

Developmental Stage	Effect of Iodine Deficiency
Prenatal development	Cretinism, deafness, short stature
Newborns and infants	Increase of mortality, abnormal brain development
Children and adolescents	Goiter and learning disabilities
Adults	Goiter, slow response time, impaired mental function
Pregnancy	Miscarriage, stillbirth, birth defects

Insufficient iodine intake is the major cause of a low iodine status. Iodine deficiency results when iodine intake is less than 20 microgram per day [3]. Iodine intake has been estimated and is highly correlated with the type (and amount) of foods consumed. Certain foods, such as seaweed and seafood, are naturally relatively rich in iodine. In addition, iodine and its compounds are used in a variety of food-related applications including nutrient fortification. Iodine fortification of a suitable food vehicle, by addition of small amount of iodine in the form of inorganic iodine (such as sodium iodide, potassium iodide, potassium iodate and copper iodide) is an efficient way to improve iodine intake and to prevent IDD, especially in people living inland who lack iodine from natural sources [4].

Today, many Asian countries choose instant noodles as food vehicle for supplementary iodine and other micronutrients. For example in Indonesia, instant noodles contain micronutrient such as vitamin A (270 μg), vitamin D (13 μg), Vitamin E (13 mg), vitamin B1 (0.3 mg), vitamin B2 (0.3 mg), vitamin B6 (3 mg), vitamin B12 (0.4 μg), niacin (200 μg), iron (8 mg), iodine (35 μg), zinc (7 mg), selenium (20 μg) and calcium (300 mg) [5]. Instant noodles can be fortified either by fortifying the flour used for making the noodle or the seasoning. Fortification of the seasoning has advantages. The fortificants are not exposed to heat and moisture during the noodle processing. In addition, the fortificants are better protected being packed in a sachet inside the pouch of instant noodles. However, interaction of nutrients-nutrients and

nutrients-ingredients may reduce the stability of the nutrients. Thailand, Indonesia and Philippines have micronutrient fortification in seasoning [6]. Instant noodles come in several different flavors which are added to the seasoning ingredient. A major ingredient is salt. The amount and type of salts varies within the seasoning flavors.

There are several methods for the determination of iodine fortification in food or food-related applications. For seasonings, colorimetric method that further development of Sveikina method by Moxon RED and Dixon EJ [8] has been used by the Division of Food Analysis, Department of Medical Sciences, Thailand for iodine determination in sea weed added snack food [9] and ICP-MS technique have been used in this country.

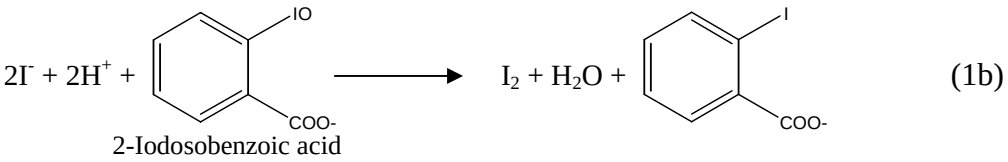
The Sveikina method is a catalytic method. Iodide catalyzes the reaction between ferrous thiocyanate and nitrite. The color of ferrous thiocyanate fades on reaction with nitrite. The net reaction is shown below (see reaction (a)). Using iodide as catalyst, the rate of color fading of ferrous thiocyanate is related to concentration of iodide, with the absorbance measurement after 20 min. The method is sensitive with good correlation coefficient to iodide.

$$2SCN^- + 3NO_2^- + 3NO_3^- \xrightarrow{I^-} 2CN^- + 2SO_4^{2-} + 6NO + H_2O \qquad \text{(a)}$$

ICP-MS is a sensitive technique for trace element determination, but precision of this technique is poor for salt matrix. The analytical precision depends on the stability of the aerosol production, ionization in the plasma, ion sampling and detection. When the salt content in the analyte solution is high, salt deposits at the nebulizer, the torch and the sampler can lead to drift in the signal. These effects lead not only to signal depression and signal fluctuation but also to memory effect. Therefore, the total salt contents of the solution should not be above 0.1-0.5 g/100 mL depending on the salt used [10].

Iodide as the iodo-derivative with 2,6-dimethyphenol, *N*,*N*-dimethylaniline or 2,6-dimethyaniline reagent was proposed for high chloride matrix such as sea water and iodized salt [11, 12, 13, 14]. Iodide is oxidized by 2-iodosobenzoate to iodine which is converted to the iodo-derivative after reacting with a derivatizing agent. The

oxidizing agent, 2-iodosobenzoate, is a selective for iodide in neutral aqueous solution. The reactions are shown below.

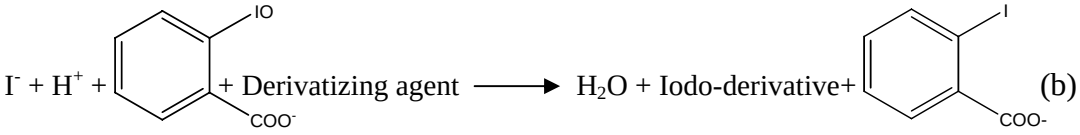


2-Iodosobenzoic acid

(1b)

I_2 + Derivatizing agent ⟶ Iodo-derivative + I^- + H^+ (2b)

Net Reaction



(b)

2-Iodosobenzoic acid

In this work, gas chromatography (GC) with ion-trap mass spectrometry (MS) will be used for iodo-derivative analysis. Gas chromatography is employed for separation of the different components within a capillary column. When the sample exits the gas chromatograph, capillary column introduces small quantities of the sample into the mass spectrometer.

Mass spectrometer determines the mass of a molecule by measuring the mass-to-charge ratio (m/z) of its ion. The result of molecular ionization, ion separation, and ion detection is a spectrum that can provide molecular mass and structural information. The ion trap mass spectrometer consists of a ring electrode and two hyperbolic endcap electrodes. A schematic of an ion trap is shown in Figure 1.1.

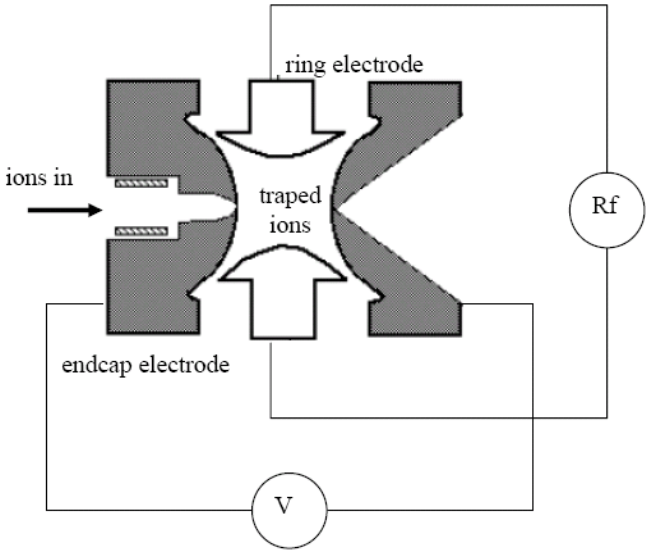


Figure 1.1 A schematic (cutaway view) of an ion trap mass analyzer.

Various voltages are applied to these electrodes resulting in the formation of a cavity in which ions are trapped. The ring electrode RF potential, an a.c. potential of constant frequency but variable amplitude, produces a 3D quadrupolar potential field within the trap. This traps the ions in a stable oscillating trajectory. The exact motion of the ions is dependent on the voltages applied and their individual mass-to-charge (m/z) ratios. For detection of the ions, the potentials are altered to destabilise the ion motions resulting in ejection of the ions through the exit endcap. The ions are usually ejected in order of increasing m/z by a gradual change in the potentials. This 'stream' of ions is focussed onto the detector of the instrument to produce the mass spectrum [15].

The advantages of the ion-trap mass spectrometer are compact size, relatively low priced and the ability to trap and accumulate ions to increase the signal-to-noise ratio of a measurement.

CHAPTER II
OBJECTIVES

This work is a study of the determination of iodide in aqeoues solution by GC-MS. The derivatization procedure and derivatizing agents will be studied. The most suitable reagent will be applied for the iodide determination in instant noodle seasonings. The objectives of this study are:

2.1 To develop a procedure for iodide determination by GC-MS.

2.2 To study four derivatizing agents, 2,6-dimethylphenol, *N*,*N*-dimethylaniline, 2,6-dimethylaniline and 2,6-diiosopropylaniline. The analytical performance in terms of reaction time, correlation coefficient (r^2) and detection limit.

2.3 To investigate the developed method for iodide determination in urine samples.

2.4 To apply the developed method for iodide determination in seasoning powders for instant noodle.

CHAPTER III
LITERATURE REVIEW

3.1 Iodide Determination

Iodide can be measured by several techniques such as colorimetric spectrometer, colorimetric titration, catalytic reaction, chemiluminescence, Inductively Coupled Plasma (ICP), Neutron Activation Analysis (NAA), Atomic Absorption Spectrometer (AAS), Electrochemistry, X-Ray Fluorescence (XRF), Liquid Chromatography (LC) and Gas Chromatography (GC).

3.1.1. Colorimetric Measurement: Iodide Complex

a) Methyleneblue-iodide Complex in 1,2-Dichloroethane

Iodide was separated from other species by oxidation and solvent extraction, followed by back-extraction into aqueous phase, to form a complex with methyleneblue and extract the ion-pair of methyleneblue-iodide into 1,2-dichloroethane. Spectrophotometric measurement of the complex was carried out at 657 nm [16].

b) Triiodide-starch Complex

Iodide was oxidized to iodine then it was separated through hydrophobic membrane and formed complex with starch. The complex was measured by spectrometry at 590 nm. The reaction applied in pharmaceutical products [17].

3.1.2 Colorimetric Measurement: Catalytic Reaction

a) Chlorpromazine (CP)-hydrogen Peroxide Reaction

The determination of traces of iodide was based on its catalytic effect on the oxidation of chlorpromazine (2-chloro-10-(3-dimethylaminopropyl) phenothiazine: $C_{17}H_{19}ClN_2S$)

$$C_{17}H1_9ClN_2S + 3H_2O_2 + 4H^+ \xrightarrow{I^-} 2C_{17}H_9ClN_2SOH^{2+} + 4H_2O \qquad (1.1)$$

Iodide accelerated the formation of the red free radical. The reaction was followed by measuring the absorbance increasing at 525 nm [18].

b) Pd^{2+} Catalyses Reaction between Co^{3+}-EDTA and the Hypophosphite

Iodide inhibited Pd^{2+} catalysed effect on reaction between EDTA-Co^{3+} and the phosphate ion [19]. Concentration of iodide was proportional to decreasing rate of the reaction. The reaction was followed by colorimetric measuring of Co^{3+} -EDTA complex at 540 nm.

c) The Sveikina Method

The Sveikina method was the catalytic effect of iodide on the decomposition of Fe^{3+} - SCN complex in the presence of NO_2^- [20].

$$2SCN^- + 3NO_2^- + 3NO_3^- + 2H^+ \xrightarrow{I^-} 2CN^- + 2SO_4^{2-} + 6NO + H_2O \qquad (1.2)$$

Colorimetric measurement at 450 nm of ferric thiocyanate was related to iodide concentration.

d) The Sandell-Kolthoff Reaction

The Sandell-Kolthoff reaction is commonly used, for determination of trace iodide, because of specificity and high sensitivity. The reaction was based on catalytic activity of iodide in the redox reaction of Ce(IV) and As(III) to Ce(III) and As(V). The reaction followed the yellow color of Ce(IV) [21, 22, 23].

$$2Ce^{4+} + As^{3+} \xrightarrow{I^-} 2Ce^{3+} + As^{5+} \qquad (1.3)$$

yellow colorless

Furthermore, some catalytic methods were developed using pseudo-first order kinetics for low concentrate inorganic iodine [24].

3.1.3 Chemiluminesecne

The iodide was converted to iodine by potassium dichromate under stirring in the closed head-space vial and the iodine was released from urine by the thermostatting. Iodine, luminol and Co(II) solution were carried by the flow injection system and mixed in front of detector which detected emission intensity at 425 nm. The application was for urine samples [25]. Another application used gas-diffusion (GD) unit for iodine separation and was applied to pharmaceutical products [26].

3.1.4 Inductively Coupled Plasma (ICP)

The isotope dilution ICP-MS has been applied to dried food samples. The long-lived nuclide ^{129}I was added to sample and $^{127}I/^{129}I$ isotope ratio was measured by ICP-MS after digestion of sample with nitric acid using a high-pressure autoclave (130 mbar) at 230 ^{o}C [27]. The other method, sample preparation was extraction of iodine by tetramethylammoniumhydroxide, TMAH solution at high temperatures in closed vessel and sample decomposition by acid mixture of perchloric acid and nitric acid using microwave [28].

Standard addition method was used for iodine determination in finely ground diet and lyophilized meat samples and measured by ICP-MS. Sample treatment was disintegration of matrix in closed-vessel at 90 ˚C for 3 hr by TMAH [29].

3.1.5 Atomic Absorption Spectrometry (AAS)

Indirect measurement of iodide in iodized salt has been determined by flame atomic absorption spectrometry (FAAS). The redox reaction of Cr(VI) and iodide to Cr(III) and iodine in acid medium has been used. Iodide concentration related to the absorbance decrease of Cr(VI). This method had an advantage of automatically continuous flow procedure [30].

3.1.6 Electrochemistry

Shuxun *et al.* proposed iodide determination in food and biological samples by cathodic stripping voltammetry using Zephiramine (Zeph) as an ionic associating agent. Samples were treated by oxygen combustion. Iodide was oxidized to iodine (I_2) then combined with bromide to give I_2Br^- and associated with Zeph to ZephI_2Br [31].

Boron-doped diamond thin film (BDD) has been used as the electrode as amperometric detector for iodide determination in nuclear emergency tablets [32].

3.1.7 X-ray Fluorescence (XRF)

Dietary supplement products were grounded and digested with 25% of ammonium hydroxide. Sample solution was diluted then analyzed by total reflection X-ray fluorescence (TXRF) [33].

3.1.8 Neutron Activation Analysis (NAA)

Iodide was preconcentrated by hydrophobic polymer inclusion sorbent (PIS) and then analyzed by neutron activation analysis (NAA) with HPGe detector. Iodate was determined in form of iodide after reduced by mixture of ascorbic acid and acetic

acid. Including, total iodine was determined by epithermal neutron activation analysis (ENAA). They were applied in milk and milk powder samples [34].

3.1.9 Liquid Chromatography (LC)

Iodide was converted to iodine, then sequestered with starch, and separated from the matrix using a Shim-pack DIOL-150 size exclusion column with methanol–0.01 mol L^{-1} aqueous phosphoric acid (10:90, v/v) as mobile phase and UV detection at 224 nm. The method was successfully applied to the determination of iodide in seawater and urine [35].

Iodide in water was separated and analyzed by high performance liquid chromatography (HPLC) using strong anion exchanger column, Dionex AS 11, with UV detector at 226 nm. Dissolved organic iodine and iodate were determined in form of iodide after sample preparation. Organic iodine was decomposed and reduced to iodine by dehydrohalogenation and $NaHSO_3$, respectively. Only reduction was necessary for iodate [36].

Iodide in seawater was preconcentrated and separated by conventional liquid chromatography (LC) on anion exchanged resin with polystyrene-divinylbenzene metric column and UV spectrometric detector at 226 nm [37].

Iodide in seawater was determined by electrostatic ion chromatography (EIC) with UV detector at 210 nm. An electrostatic ion chromatographic column was modified by coating ODS pack column with Zwittergent-3-14 micelles [38].

3.1.10 Gas Chromatography (GC)

For Gas Chromatography technique, non-volatile iodide has been converted to volatile species which can be separated and analyzed by GC.

In 1974, Grys ST. published a method for the inorganic iodine determination in milk. After protein precipitation by sulfuric acid, iodide was oxidized with iodate in the presence of acetone followed by an extraction of iodoacetone with n-hexane and then analysed by GC-ECD [39].

$$2I^- + IO_3 + 3H^+ + 3CH_3COCH_3 \longrightarrow 3ICH_2COCH_3 + 3H_2O \qquad (1.4)$$

GC-FID have been employed to determine iodide in form of 2-iodoethanol. The conversion of iodide to 2-iodoethanol was reaction of iodide and olefin oxide in acidic solution [40].

$$I^- + CH_2OCH_2 + H^+ \longrightarrow ICH_2CH_2OH \qquad (1.5)$$

Aqueous inorganic iodide determination in form of alkyl iodide by GC-TCD was proposed in 1970 by McGee J and Allen KG. Alkylation of aqueous inorganic iodide by the thermal degradation of its tetraalkylammonium salt gave alkyl iodide and trialkylamine. Alkyl iodide was seperated on porous polymer column of GC and detected by thermal conductivity detector [41].

$$I^- + R_4N+ \longrightarrow R_4NI \qquad (1.6)$$

$$R_4NI \longrightarrow RI + R_3N \qquad (1.7)$$

Where R = alkyl group

Iodide in water, salt, cow milk and human blood serum were determined by GC-ECD after derivatization with acetone. Cow milk and human blood serum sample were deproteinized before derivatization. The method was the oxidation of iodide in the presence of acetone to iodoacetone by chromate and permanganate then single extraction of the derivative by benzene [42].

Iodide converts to iodine by reaction of iodide ion and iodate ion in acidic solution then extracted into carbon tetrachloride. GC-TCD were used to analyze iodine in carbon tetrachloride [43].

$$2I^- + IO_3 + 3H^+ \longrightarrow 3I_2 + 3H_2O \qquad (1.8)$$

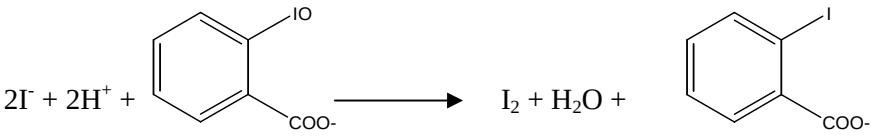
3.2 Chromatographic Determination of Iodide by Oxidation with 2-Iodosobenzoate and Derivatization with Phenol or Aniline

Chromatographic technique has been employed to analyze iodide after oxidation with 2-iodosobenzoate and iodination (derivatization) with phenol or aniline.

2-Iodosobenzoate was selective oxidizing agent for iodide in neutral or acid free solution. Aromatic phenol and aromatic amine underwent electrophilic aromatic substitution reaction. Hydroxyl and amine group on aromatic rings were very strongly activating substituents classified as ortho- and para-direction [44]. Iodination with 2,6-dimethylphenol or 2,6-dimethylaniline gave only one product, 4(para)-iodo-2,6-dimethylphenol or 4(para)-iodo-2,6-dimethylaniline, respectively.

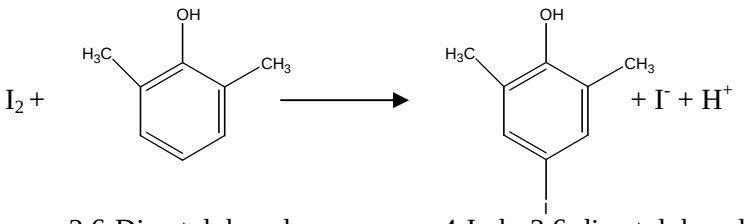
In 1992, Verma *et al.* used 2-iodosobenzoate for oxidation of iodide to iodine and derivatization with 2,6-dimethylphenol. Precolumn derivatization of iodide by 2,6-dimethylphenol was determined by high performance liquid chromatography (HPLC) using ODS column with UV detector at 220 nm. Natural water, sea water, iodized salt, milk and pharmaceuticals sample were applied [11, 45].

Shin *et al.* developed this method for GC-MS, reported in 1996. Iodide was oxidized by 2-iodosobenzoate to iodine. It was derivatized with 2,6-dimethylphenol then converted to 4-iodo-2,6-dimethylphenol. A 20 min reaction time was reported for derivatization. Derivative was extracted by diethyl ether then measured by GC-MS. This technique gave limit of detection in ng L^{-1} level [12]. The reactions are shown below.



(2.1)

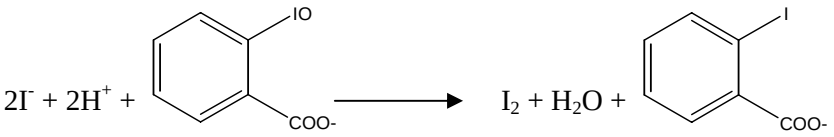
2-Iodosobenzoic acid



(2.2)

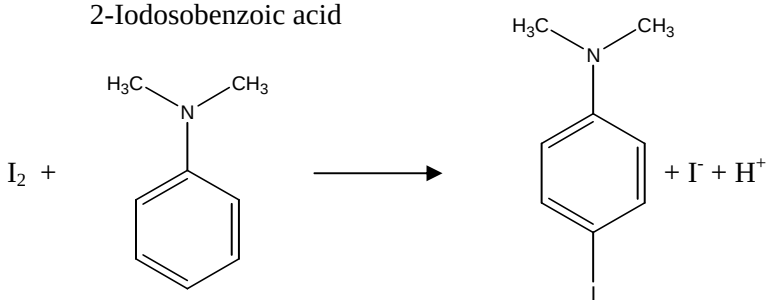
2,6-Dimetylphenol 4-Iodo-2,6-dimetylphenol

In 2000, Mishra *et al.* proposed aromatic amine, *N*,*N*-dimethylaniline as derivatizing agent. 2-iodosobenzoate oxidized iodide to iodine. It was converted to 4-iodo-*N*,*N*-dimethylaniline by derivatization with *N*,*N*-dimethylaniline. Derivatization of *N*,*N*-dimethylaniline was complete within 1 min. The derivative was extracted by cyclo-hexane then measured by GC-MS [13]. The reactions are shown below.



(2.3)

2-Iodosobenzoic acid



(2.4)

N,*N*-dimetylaniline 4-Iodo- *N*,*N*-dimetylaniline

Other application of *N*,*N*-dimethylaniline derivatization was determination of iodine in pharmaceuticals, iodized salt, milk powder and vegetables. Solid phase microextraction (SPME) and single drop microextraction (SDME) with iso-octane has been used for iodo-derivative extraction [46].

Reddy-Noone *et al.* used the other aromatic amine, 2,6-dimethylaniline, as derivatizing agent. This method has been applied to seawater and table salt. Iodide and iodate, after reduction to iodide with ascorbic acid, were converted to 4-iodo-2,6-dimethylaniline by reaction with 2-iodosobenzoate in the presence of 2,6-dimethylaniline. Derivatization of 2,6-dimethylaniline at pH 6.4 was complete within 10 min. Single drop microextraction (SDME) and liquid-phase microextraction (LPME) in toluene have been employed for iodo-derivative extraction and then analyzed by GC-MS and GC-FID [14].

3.3 Iodide Determination in Iodine Fortified Food Product

Kamlert *et al.* determined iodine in sea weed added snack food by Moxon and Dixon colorimetric. Sample preparation was alkaline ash at 550˚C for destruction of organic matter. The residue was dissolved in 50±0.5 mL and centrifuge at 3000 rpm for 5 min. A 5 mL of supernatant was analyzed iodide by catalytic reaction of ferric complex and nitrite. Ferric complex decoloration was measured at 450 nm after 20 min [9].

Perring *et al.* re-evaluated the original Moxon and Dixon colorimetric method for more robustness, suitable to routine work. The rapid rate of decoloration was the major problem for poor precision. Decoloration rate has been reduced by dilution of sodium nitrite solution 100 times. Iodide and iodate were determined in fortified culinary products containing high salt by Moxon and Dixon colorimetric method. Sample preparation was dissolution of iodide and iodate in hot water (60°C) and stirred for 30 min. Then the sample was allowed to cool down to room temperature and was made up to 100 mL with water. The product solution was well mixed and filtered through a 0.2 μm membrane filter. The reaction was prepared for 60 min at 60±2 °C in a water bath then cooled in ice water for 10 min. The destruction of ferric-thiocyanate was followed by spectrophotometry at 454 mn [47].

CHAPTER IV
EXPERIMENTAL

In this chapter, an instrumental description, operating conditions used in the analytical measurements, standard and reagent solutions preparation, and experimental procedures are presented.

4.1 Instrumentation

The following is the list of equipments used in this work.

4.1.1 The GC-MS Instrument

The GC-MS consist of the following:

Auto sampler model	Thermo Finnigan AS2000 (USA)
GC model	Thermo Finnigan TRACE GC 2000 (USA)
MS model	Thermo Finnigan PolarisQ (ion trap mass detector) (USA)
Software	Xcalibur version 1.4

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Table 4.1 GC-MS condition for four different iodo- derivatives

Injection volume	1 μL
Injection mode	Splitless
Splitless time	1 min
Injector temp	280 ˚C
Oven temp	60 ˚C (1 mim) --15 ˚C/min--> 210 ˚C--30˚C/min--> 300 ˚C(3 min) for iodo-2,6-dimethylphenol 90 ˚C (3 mim) --30 ˚C/min--> 210 ˚C(3 min) for iodo-*N*,*N*-dimethylaniline 90 ˚C (3 mim) --30 ˚C/min--> 130 ˚C--20 ˚C/min-->150 ˚C--3˚C/min -->165 ˚C --30 ˚C/min--> 200 ˚C for iodo-2,6-dimethylaniline 90 ˚C (3 mim) --20 ˚C/min--> 180 ˚C--5 ˚C/min-->200 ˚C(3 min) for iodo-2,6-diisopropylaniline
Transfer line temp	300 ˚C
He flow rate	1.00 mL/min (99.999%)
Scan mode	50-350 *m/z*, quantitation at 248 *m/z* for iodo-2,6-dimethylphenol and 247 *m/z* for iodo-*N*,*N*-dimethylaniline 50-450 *m/z*, quantitation 247 *m/z* for iodo-2,6-dimethylaniline and at 303 *m/z* for iodo-2,6-diisopropylaniline
Ionization mode	EI, 70eV (200 ˚C)

4.1.2 The ICP-MS Instrument

Model	PerkinElmer Elan 6000 (USA)
Software	Elan instrument software version 3.3

Table 4.2 ICP-MS operation condition

Analyte	I (127)
Internal standard	Rh (103)
Plasma gas flow[a]	15 L/min
Auxiliary gas flow[a]	0.8 L/min
Nebulizer gas flow[a,b]	1.0 L/min
RF power	1150 W

[a] Ar gas was used

[b] Nebulizer type : Cross-flow

4.1.3 Analytical Balance

Model	Sartorius CP225D (Germany)

4.1.4 Aspirator and Vacuum tank

Model	Eyela A35 (Japan)

4.1.5 Autopipette

Model	Eppendorf Research (Germany)
Volume	2-20, 20-200 and 100-1000 μL
Model	Brand (Germany)
Volume	0.5-5.0 mL

4.1.6 Centrifuge

Model	Sanyo Centure2 (UK)

4.1.7 Furnace

Model	Lindberg 51524 (USA)

4.1.8 Magnetic Stirrer

Model	Heidolph MR 3000 and MR3001 (Germany)

4.1.9 pH Meter

Model	Thermo Orion 420A+ (USA)

The glass combination with reference electrode is model 9157 from Thermo Orion (USA). Standard buffer solutions of pH 4.00±0.01, 7.00+0.01 and 10.00+0.01 are from Merck (Germany).

4.1.10 Shaker

Model	3006GFL by Gesellschaft Fürlaboratechnik MbH (Germany)

4.1.11 Spectrophotometer

Model	PerkinElmer Lambda35 (USA)
Software	UV Winlab version 2.85.04

4.1.12 Vortex

Model	Vortexgenie2^{TM} G560 by Scientific Industries (USA)

4.2 Materials

4.2.1 GC Column

GC column is ZB-5 (cross-linked 5% phenylpolylsiloxane) 30 m ^{x} 0.32 mm i.d., 0.25 μm film thickness

4.2.2 Cuvette

Starna® cuvette glass windows (12.5 x 12.5 x 45 mm), 10 mm path length, 3.500 mL nominal volume (USA) was used for colorimetric based on Sveikina method in the procedure a) of Section 4.5.7.

4.2.3 Filter

Nylon filter membranes (0.45 μm pore size, 47 mm i.d.) supplied by Agilent Technologies (Germany) were used for sodium-2-iodosobenzoate preparation in Section 4.4.4.5.

Mixed cellulose (CA-CN) filter membrane disposable devices (0.2 μm pore size, 13 mm i.d.) supplied by Restek corporation (USA) were used for instant noodle seasoning preparation for ICP-MS in procedure b) Section 4.5.7.

MN paper filters (11.5 cm i.d.) were used for instant noodle seasoning preparation for GC-MS in Section 4.4.8.

4.3 Reagents

Chemicals in this work were AR grade. Organic solvent are HPLC or pesticide grade. Deionized-distilled water (DI water) (18.2 MΩ•cm^{-1}), obtained from a Milli-Q-system, was used for preparation of reagent solutions. List of chemicals and their suppliers is shown in Table 4.3.

Table 4.3 List of chemicals and their suppliers

Chemical	**Formula**	**Supplier**
Potassium iodide	$KI_{(s)}$	E. Merck(Germany)
Potassium dihydrogen phosphate	$KH_2PO_{4(s)}$	E. Merck(Germany)
diPotassium hydrogen phosphate	$K_2HPO_{4(s)}$	E. Merck(Germany)
Certified iodide standard solution	$KI_{(aq)}$	RTC(USA)
2-iodosobenzoic acid	$C_7H_5IO_{3(s)}$	Sigma-Aldrich(USA)
2,6-Dimethylphenol	$C_8H_{10}O_{(s)}$	Sigma-Aldrich(USA)
N,*N*-dimethylaniline	$C_8H_{11}N_{(l)}$	Fluka(Switzerland)
2,6-Dimethylaniline	$C_8H_{11}N_{(l)}$	Acros
2,6-Diisopropylaniline	$C_{12}H_{19}N_{(l)}$	Acros
2,4,6-Trichlorophenol	$C_6H_3Cl_3O_{(s)}$	E. Merck(Germany)
3,4,α-Trichlorotoluene	$C_7H_5Cl_{3(s)}$	Fluka(Switzerland)
Diphenylamine	$C_{12}H_{11}N_{(s)}$	E. Merck(Germany)
Sodium hydroxide	$NaOH_{(s)}$	E. Merck(Germany)
Sodium nitrate	$NaNO_{3(s)}$	Lab Scan(Thailand)
Methyl-*tert*-butyl ether	$CH_3O(CH_3)_3$	Lab Scan(Thailand)
Methanol	$CH_3OH_{(l)}$	Lab Scan(Thailand)
n-Hexane	$C_6H_{16(l)}$	Lab Scan(Thailand)
Nitric acid	$HNO_{3(l)}$	Lab Scan(Thailand)
Sep-Pak Cartridge	C18	Waters(USA)
Strong anion exchange Cartridge		Altech(USA)

4.4 Preparations of Standard Solutions and Reagents

The following sections are procedures for preparation of standard solutions and reagent solution which employed in this work.

4.4.1 Stock Standard Iodide Solution (1000 μg I/mL)

Dry potassium iodide (105˚C over night) was weighed accurately for 0.1307 g and then dissolved and diluted to 100 mL in a volumetric flask with DI water. The stock solution was kept in a plastic bottle and storage in refrigerator.

4.4.2 Intermediate Standard Iodide Solution (1 μg I/mL)

A 100 μL aliquot of stock standard iodide solution was diluted to 100 mL in a volumetric flask with DI water. The solution was kept in a plastic bottle and storage in refrigerator. Working standards of iodide were prepared by appropriate dilution with water.

4.4.3 Certified Standard Solution (1 μg I/mL)

A 100 μL aliquot of 1000 μg I/mL certified standard solution was placed in 100 mL volumetric flask and made up to volume with DI water. Certified standard solution (1 μg I/mL) was kept in a plastic bottle and storage in refrigerator.

4.4.4 Derivatization Reagents

4.4.4.1 2,6-Dimethyphenol Solution (2500 μg/mL in MeOH)

2,6-Dimethyphenol was weighed 12.5 mg and dissolved and diluted to 5 mL with methanol. The solution was kept in refrigerator.

4.4.4.2 *N,N*-dimethylaniline Solution (190 μg/mL in MeOH)

A 20 μL aliquot of *N,N*-dimethylaniline (99.5%, d = 0.956) was placed in 100 mL volumetric flask and made up to volume with methanol. The solution was kept in cool place.

4.4.4.3 2,6-Dimethylaniline Solution (194 μg/mL in MeOH)

A 20 μL aliquot of 2,6-dimethylaniline (99%, d = 0.980) was placed in 100 mL volumetric flask and made up to volume with methanol. The solution was kept in cool place.

4.4.4.4 2,6-Diisopropylaniline Solution (259 μg/mL in MeOH)

A 30 μL aliquot of 2,6-dimethylaniline (92%, d = 0.940) was placed in 100 mL volumetric flask methanol. The solution was kept in cool place.

4.4.4.5 Sodium-2-iodosobenzoate Reagent

2-Iodosobenzoic acid (1 g) was stirred in 20 mL of 0.2 M NaOH until dissolved. Solution was diluted to 250 mL with DI water and filtered through a 0.45 μm membrane filter. The solution was stable at least 4 months at room temperature.

4.4.4.6 Phosphate Buffer Solution, pH 6.4

Potassium dihydrogen phosphate (20 g) and dipotassium hydrogen phosphate (20 g) were dissolved with DI water and mixed. Mixed solution was made up to 500 mL volume with DI water. Phosphate buffer solution was adjusted to pH 6.4. The solution was kept in refrigerator.

4.4.4.7 2,4,6-Trichlorophenol Solution (8.25 μg/mL in MeOH)

2,4,6-Trichlorophenol was weighed 82.5 mg and dissolved in 10 mL methanol as stock solution. A 10 μL aliquot of 2,4,6-trichlorophenol stock solution was diluted with methanol to 10 mL volume. Solution was kept in refrigerator.

4.4.4.8 Diphenylamine Solution (62.5 μg/mL in MeOH)

A 625 μL aliquot of 1 mg/mL diphenylamine solution was diluted to 10.0 mL with methanol. The solution was kept in refrigerator.

4.4.4.9 3,4,α-Trichlorotoluene Solution (796 μg/mL in MeOH)

A 5.82 mL aliquot of 3,4,α-trichlorotoluene (97%, d=1.41) was diluted to 10 mL with methanol. The solution was kept in refrigerator.

4.4.5 Sample Cleanup Reagent

4.4.5.1 Sodium Nitrate Solution (3 M)

Sodium nitrate (25.497 g) was dissolved and made up to 100 mL volume with DI water. The solution used for iodide elution from SAX.

4.4.6 ICP-MS Reagent

4.4.6.1 Rhodium Internal Standard Solution (1 mg/L Rh)

The working solution was prepared by dilution of 100 μL of the Rhodium reference standard solution of 1000 mg Rh/L to 100.0 mL with 2% nitric acid.

4.4.7 Sveikina Reagents

4.4.7.1 Potassium Carbonate Solution (30% m/V)

Potassium carbonate (30 g) was dissolved and made up to 100 mL volume with DI water.

4.4.7.2 Zinc Sulfate Solution (10% m/V)

Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$) was weighed 10 g and dissolved in 100 mL of DI water.

4.4.7.3 Potassium Thiocyanate Solution (0.023% m/V)

Potassium thiocyanate (0.23 g) was dissolved and made up to 1 L with DI water.

4.4.7.4 Sodium Nitrite Solution (2.07% m/V)

Sodium nitrite (2.07 g) was dissolved and made up to 100 mL volume with DI water. The solution was daily prepared.

4.4.7.5 Ammonium Iron (III) Sulfate Reagent

Ammonium iron (III) sulfate ($NH_4Fe(SO_4)_2 \cdot 12H_2O$) was weighed 77 g and dissolved in approximately 400 mL of DI water. A 167 mL aliquot of nitric acid was added in ammonium iron (III) sulfate solution. The reagent was diluted to 1 L with DI water.

4.5 Procedures and Methods

The procedures of the analytical methods for iodide determination after derivatization with 2,6-dimethylphenol, *N*,*N*-dimethylaniline, 2,6-dimethylaniline and 2,6-Diisopropylaniline, the study of internal standard and reaction time, sample preparation, quantitative determination of iodide by GC-MS and comparison methods are presented in the following sections.

4.5.1 The Analytical Procedures of Iodide Determination after Derivatization

a) 2,6-Dimethylphenol as Derivatizing Agent

A 3 mL of standard solution of iodide (1 ppm) was pipetted into test tube. A 2 mL of phosphate buffer, 20 μL of 2,6-dimethylphenol and 1 mL of sodium-2-iodosobenzoate were added and shook with shaker for 20 min at room temperature. After that, 40 μL of 2,4,6-trichlorophenol and 1 mL of methyl-*tert*-butyl-ether were added. Solution was extracted into methyl-*tert*-butyl-ether layer by shaking for 15 min and centrifuging for 5 min at 1500 rpm. 1 μL of methyl-*tert*-butyl-ether portion was injected to GC-MS.

b) *N*,*N*-dimethylaniline as Derivatizing Agent

A 3 mL of standard solution of iodide (200 ppb) was pipetted into test tube. A 0.5 mL of phosphate buffer, 0.5 mL of *N*,*N*-dimethylaniline and 0.4 mL of sodium-2-iodosobenzoate were added and shook with vortex for 1 min at room temperature. After that, 40 μL of diphenylamine and 1 mL of n-hexane were added. Solution was extracted into n-hexane layer by shaking for 20 min and centrifuging for 5 min at 1500 rpm. 1 μL of n-hexane portion was injected to GC-MS.

c) 2,6-Dimethylaniline as Derivatizing Agent

A 3 mL of standard solution of iodide (250 ppb) was pipetted into test tube. A 0.5 mL of phosphate buffer, 0.5 mL of 2,6-dimethylaniline and 0.4 mL of sodium-2-iodosobenzoate were added and shook with shaker for 10 min at room temperature. After that, 40 μL of diphenylamine and 1 mL of n-hexane were added. Solution was extracted into n-hexane layer by shaking for 20 min and centrifuging for 5 min at 1500 rpm. 1 μL of n-hexane portion was injected to GC-MS.

d) 2,6-Diisopropylaniline as Derivatizing Agent

A 3 mL of standard solution of iodide (250 ppb) was placed into test tube. A 0.5 mL of phosphate buffer, 0.5 mL of 2,6-diisopropylaniline and 0.4 mL of sodium-2-iodosobenzoate were added and shook with shaker for 20 min at room temperature. After that, 40 μL of diphenylamine and 1 mL of n-hexane were added. Solution was extracted into n-hexane by shaking for 20 min and centrifuging for 5 min. 1 μL of n-hexane portion was injected to GC-MS.

4.5.2 Internal Standard Study

This section is study of appropriate internal standard for analysis of iodo-derivative by considering the relation of the area ratio of iodo-derivative to internal standard and iodide concentration in range of 10-250 ppb of 3 mL standard solution.

a) 2,4,6-Trichlorophenol

The study of 2,4,6-trichlorophenol as internal standard in 2,6-dimethylphenol derivatization was followed Section 4.5.1a). A 1 ppm of iodide standard solution was replaced by 10-250 ppb iodide standard solution.

b) 3,4,α-Trichlorotoluene

Using 3,4,α-trichlorotoluene replaced 2,4,6-trichlorophenol in 2,6-dimethylphenol derivatization. 3,4,α-Trichlorotoluene preparation was followed in Section 4.4.4.9. The procedure was followed Section 4.5.1a). The concentration of iodide standard solution in the procedure was replaced by 10-250 ppb iodide standard solution.

c) Diphenylamine

For the study of *N,N*-dimethylaniline and 2,6-dimethylaniline using diphenylamine as internal standard followed Section 4.5.1b) and Section 4.5.1c). An iodide concentration in the procedure was replaced by 10-250 ppb iodide standard solution.

4.5.3 Time Period for Derivation

a) Preliminary Study

N,N-dimethylaniline was used as the derivatizing agent by Mishra *et al.*[13]. In their procedure the sample solution was prepared in 25 mL volumetric flask and extracted with 200 μL of cyclo-hexane. The derivatization time was 1 min.

Following Mishra *et al.* procedure, a 3 mL of 250 ppb iodide standard solution was mixed with reagents in 25 mL volumetric flask, swirled well and kept for various time intervals (1-600). Internal standard, a 40 μL of 62.5 ppm diphenylamine was added and the solution was diluted to the mark with DI water. A 200 μL portion of n-hexane was added, the flask shaken vigorously, allowed standing for the n-hexane layer to separate in the neck of the flask. A 1 μL aliquot of n-hexane was injected into the GC-MS.

b) Time Period for Derivation Study

Time period was studied by varying reaction time in range of 1-60 min. Time counting started after addition of sodium-2-iodosobenzoate. The solution was shook during counting.

4.5.4 Analytical Performance of Iodide Determination

The analytical performance in terms of linearity, detection limit, precision and accuracy were investigated.

a) Working Range and Linearity

Working range of iodide determination was investigated in 10-250 ppb of 3 mL aqueous solution and extracted with 1 mL organic solvent. Linearity was represented in term of correlation coefficient (r^2) of calibration graph that plotted the area ratio of iodo-derivative to internal standard relate to concentration of iodide.

b) Limit of Detection (LOD)

The limit of detection of an analyte described as concentration which gave an instrument signal significantly different from the blank signal. The limit of detection on this work was defined as a signal equal to the blank signal plus three standard deviations of the blank [48].

c) Accuracy and Precision

Certified standard solution in concentration of 100 ng I/mL was prepared from 1 μg/mL of certified standard solution. Six solutions of 100 ng I/mL certified standard solution was determined iodide using procedure b) Section 4.5.1. Accuracy was proved by statistical T-test. Precision was relative standard percentile of replicates (n=6).

4.5.5 Sample Cleanup Study with Solid Phase Extraction(SPE)

Sep-Pak (C18) and strong anion exchange (SAX) were studied as cleanup method. Using cleanup method, iodide recovery in aqueous and seasoning solution was determined. An aqueous standard solution was a 1 mL aliquot of 1 ppm iodide standard solution. Seasoning sample which preparation followed Section 4.5.6) using a 1 mL of sample solution and a mixture of 1 mL of sample solution and 1 mL of 1 ppm of iodide standard solution were original and spiked sample, respectively.

They were cleanup with Sep-Pak (C18) and SAX then diluted to appropriate volume. The diluted solutions were determined iodide using procedure b) Section 4.5.1.

a) Sep-Pak, C18

Sep-Pak (C18) reverse phase in 500 mg cartridge. Before use, cartridge was activated and equilibrated with 3 mL of methanol and 2 mL of water, respectively.

b) Strong Anion Exchange (SAX)

SAX is quaternary ammonium ion in 500 mg cartridge. Before use, cartridge was activated with 5 mL deionized water**.**

4.5.6 Iodide Determination in Urine Sample

Urine sampleswere diluted to appropriate volume with distillated water and then were centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to the new clean test tube for sample cleanup and derivatization steps. Iodide content was calculated from external calibration curve and standard addition methods.

4.5.7 Iodide Determination in Seasoning Sample

A sachet of instant noodle seasoning was dissolved in 50.00 mL DI water. After stirring for 30 min, the solution was filtered through filter paper. Filtrate was collected for analysis.

Table 4.4 Seasoning samples from list of instant noodle were used in this work

Sample		**Code**	**Labeled iodide content/pack (μg)**
Brand	**Flavor**		
MAMA	Yen ta four	A	50
MAMA	Moo sub	B	50
MAMA	Tom yum kung	C	50
MAMA	Pat kee moul	D	50
WAIWAI	Hoi lai pat cha	E	75

Using standard addition method, a sample was analyzed in 5 portions. A sample portion consists of suitable volume (100-500 μL) of sample solution, 1 μg I/mL standard solution in volume of 20, 40, 80, 120, 160 or 200 μL and deionized water for volumetric adjustment to 3 mL. Iodide content was calculated from X-intercept of extrapolated linear curve.

4.5.8 Analytical Performance of Seasoning Application

The analytical performance of iodide determination in instant noodle seasoning by GC-MS was proved by comparison with other methods as colorimetry based on Sveikina method and inductively couples plasma–mass spectrometry (ICP-MS).

a) ICP-MS Method

Sample preparation from Section 4.5.6 was filtered through 0.2 μm cellulose membrane filter for ICP-MS measurement. A 50 or 100 μL of filtrate and 100 μL rhodium nitrate (1 μg Rh/mL) were diluted to 10 mL with deionized water. Rhodium was used as internal standard for non-spectral interference and signal instability correction. The calibration curve was obtained by plotting signal ratio of iodide to rhodium relate to iodide concentration in range 2.5-25 ppb

b) The Colorimetry based on Sveikina Method

Samples were prepared by adding 1 mL of 30% m/V potassium carbonate. A 1 mL of 10% m/V zinc sulfate solution into crucible contained 5 mL each of sample solution (from Section 4.5.6) and mixed. The mixture was dried on hot plate until there was no smoke. It was then covered with lid and placed in a muffle furnace at 100˚C. The furnace temperature was evenly raised to 550˚C and maintained for 90 min. The cooled ash transferred to a centrifuge tube with 25.00 mL of water and spun at 3000 rpm for 5 min. Sample blank was prepared in the same method without sample solution.

The standard solutions were contained 0, 2, 4, 8, 12 and 16 ng I/mL and the same amount of potassium carbonate as sample solution.

Pipette 5 mL of standard solution/sample solution/sample blank into test tube, 1 mL of 0.023% potassium thiocyanate solution and 2 mL of ammonium iron (III) sulfate reagent were added. The solution was mixed on vortex. At exactly 90 sec intervals, 1 mL of sodium nitrite solution was added into a sample solution and well mixing on vortex. After 20 min, the absorbance measured at 450 nm with a spectrophotometer.

CHAPTER V
RESULTS AND DISCUSSION

5.1 Preliminary Data using 2,6-Dimethylphenol as Derivatizing Agent

The procedure and GC-MS condition as given by Shin *et al.* [12] were followed (see in Appendix A). However, the organic solvent for extraction was changed from diethyl ether to methyl-*tert*-butyl ether and no internal standard was added. The chromatogram of a sample of 1000 ppb potassium iodide solution is shown in Figure 5.1.

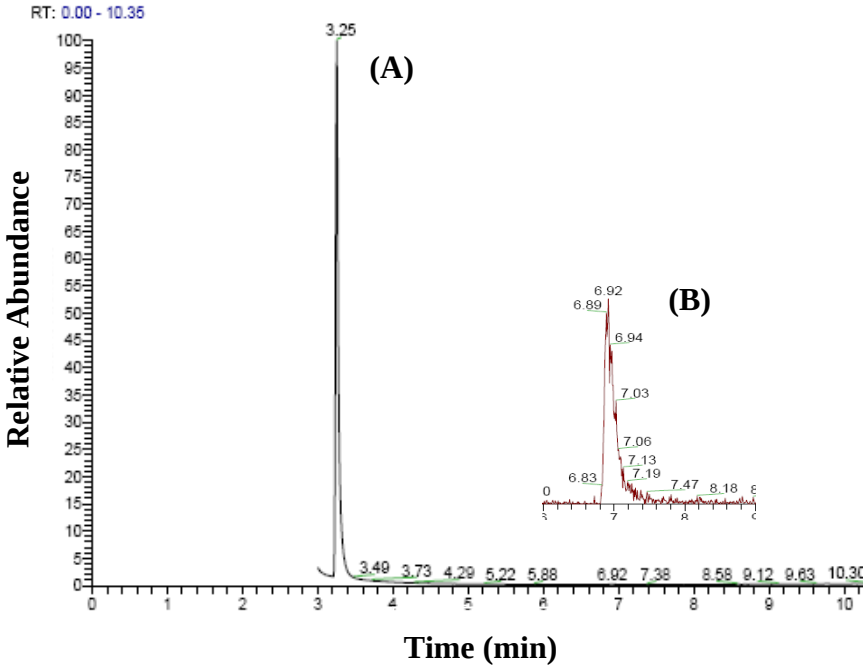

Figure 5.1a Total ion chromatogram (TIC) of the derivative (1000 ppb iodide, 3 mL) : (A) 2,6-dimethylphenol (derivatizing agent); (B) 4-iodo-2,6-dimethylphenol (derivative)

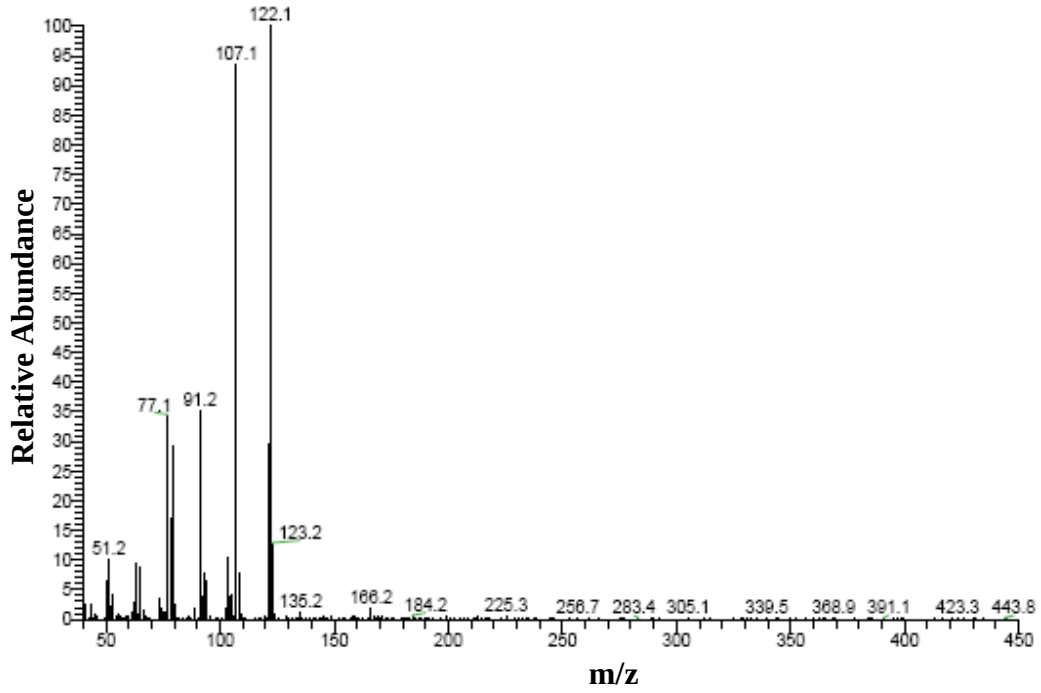

Figure 5.1b Full scan mass spectrum (*m/z* 50-450) of (A) 2,6-dimethylphenol (derivatizing agent)

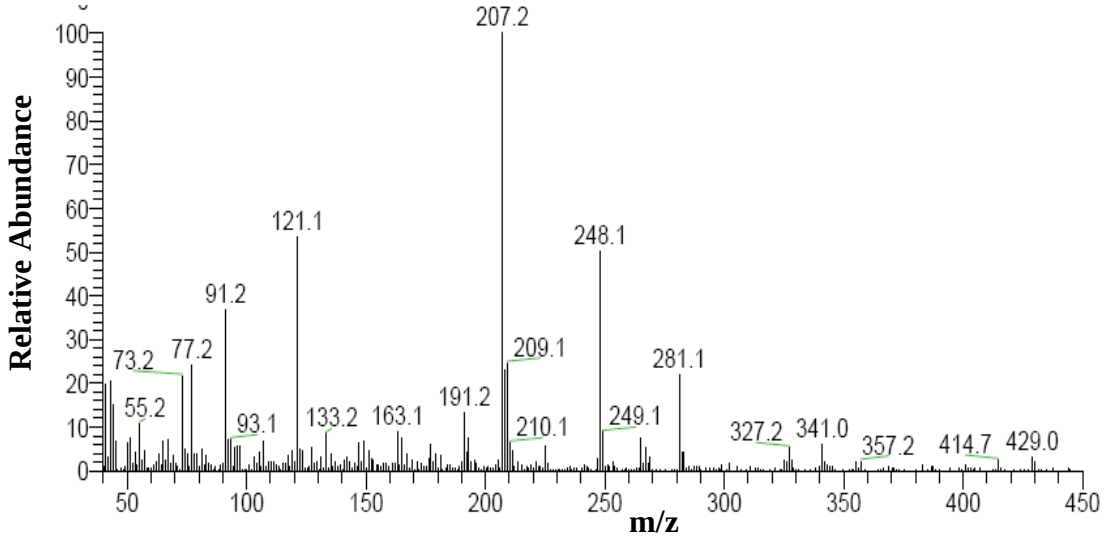

Figure 5.1c Full scan mass spectrum (*m/z* 50-450) of (B) 4-iodo-2,6-dimethylphenol (derivative)

2,6-Dimethylphenol (derivatizing agent) and 4-iodo-2,6-dimethylphenol (the iodo-derivative) were observed in the chromatogram. They have RT 3.25 and 6.92 min, respectively. The mass spectrum of 2,6-dimethylphenol shows molecular ion *m/z* 122 and 4-iodo-2,6-dimethylphenol showed molecular ion *m/z* 248. Peak shape of the derivatizing agent was very good, but peak of the iodo-derivative has poor response for 3 mL of 1000 ppb iodide standard solution.

Therefore, iodide determination by GC-MS after derivatization with 2,6-dimethylphenol can be carried out. However, the procedure and GC-MS condition need to be developed for better sensitivity.

5.2 GC-MS Condition

Chromatographic condition is important for separation by chromatographic technique. Beside separation of analyte from interference, time saving, good peak shape and good sensitivity come from suitable condition. For GC-MS, GC condition means carrier gas flow rate, injector temperature and oven temperature program. MS condition means ion source temperature and scanning range of mass spectrum which should be covered analyte molecular *m/z*.

From preliminary test, GC-MS condition for iodo-2,6-dimethylphenol was adjusted until the chromatogram showed good peak shape and good sensitivity of analyte. Injection mode was changed from split with a splitting ration of 1:10 to splitless and injection volume was decreased from 2 μL to 1 μL. Initial oven temperature program was adjusted from 100˚C and hold for 2 min to 60˚C and hold for 1 min. Mass spectrometer in full scan mode range 40-450 *m/z* was replaced with 50-350 *m/z* which cover iodo-2,6-dimethylphenol molecular *m/z* at 248. Optimal conditions are shown in table 4.1.

5.3 Total Ion Chromatogram and Mass Spectrum

GC-MS condition and procedure from table 4.1 and Chapter 4.4 were followed. Following the procedure, oxidation and derivatization reaction can be done (see reaction 1b and 2b in chapter 1). Iodide can be oxidized to iodine then converted to iodo-derivative after reacted with derivatizing agent. The derivatizing agents; as 2,6-dimethylphenol, *N*,*N*-dimethylaniline, 2,6-dimethylaniline and 2,6-diisopropylaniline, and the iodo-derivatives as 4-iodo-2,6-dimethylphenol, 4-iodo-*N*,*N*-dimethylaniline, 4-iodo-2,6-dimethylaniline and 4-iodo-2,6-diisopropylaniline can be separated and detected by GC-MS.

5.3.1 Iodide Determination using 2,6-Dimethylphenol as Derivatizing Agent and 3,4,α-Trichlorotoluene as Internal Standard

2,6-Dimethylphenol (excess derivatizing agent), 3,4,α-trichlorotoluene (internal standard, istd) and 4-iodo-2,6-dimethylphenol (iodo-derivative) were detected at 6.09, 8.91 and 10.01 min, respectively. 2,6-Dimethylphenol and 4-iodo-2,6-dimethylphenol show the molecular ion as the base ions, *m/z* 122 and 248, respectively. The 3,4,α-trichlorotoluene mass spectrum shows characteristic natural isotope ratio of chlorine at *m/z* 194, 196 and 198, respectively. Total ion chromatograms and mass spectra are shown in Figure 5.2a, 5.2b, 5.2c and 5.2d, respectively.

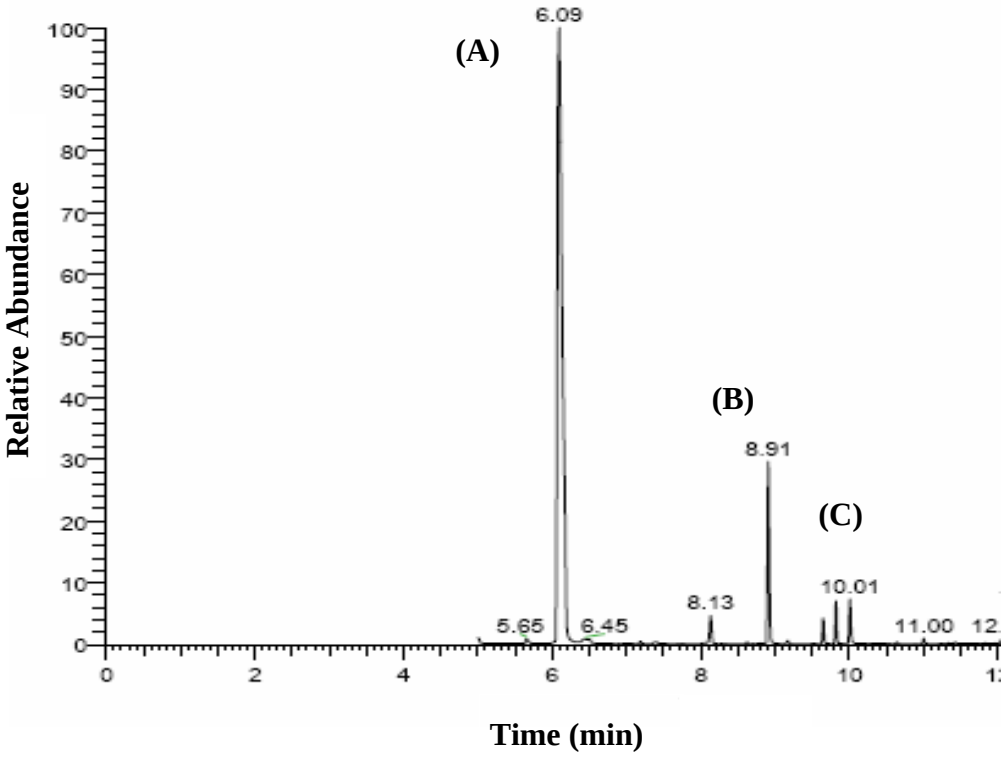

Figure 5.2a Total ion chromatogram (TIC) of the derivatives (1000 ppb iodide 3 mL, 1 mL MTBE extraction) : (A) 2,6-dimethylphenol (derivatizing agent); (B) 3,4,α-trichlorotoluene (istd); (C) 4-iodo-2,6-dimethylphenol (derivative)

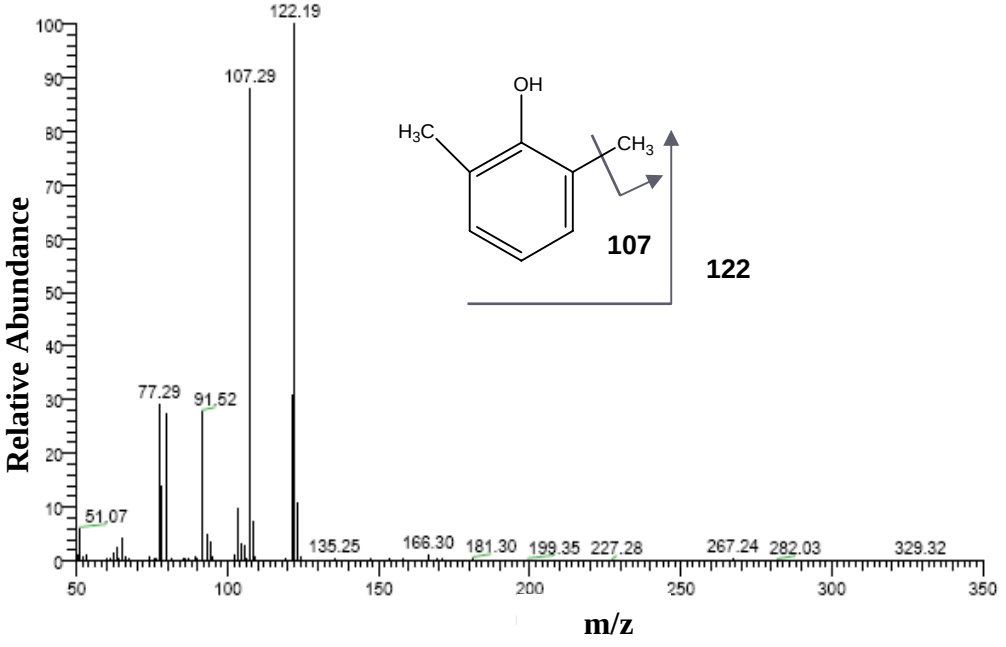

Figure 5.2b Full scan mass spectrum (*m/z* 50-350) of (A) 2,6-dimethylphenol (derivatizing agent)

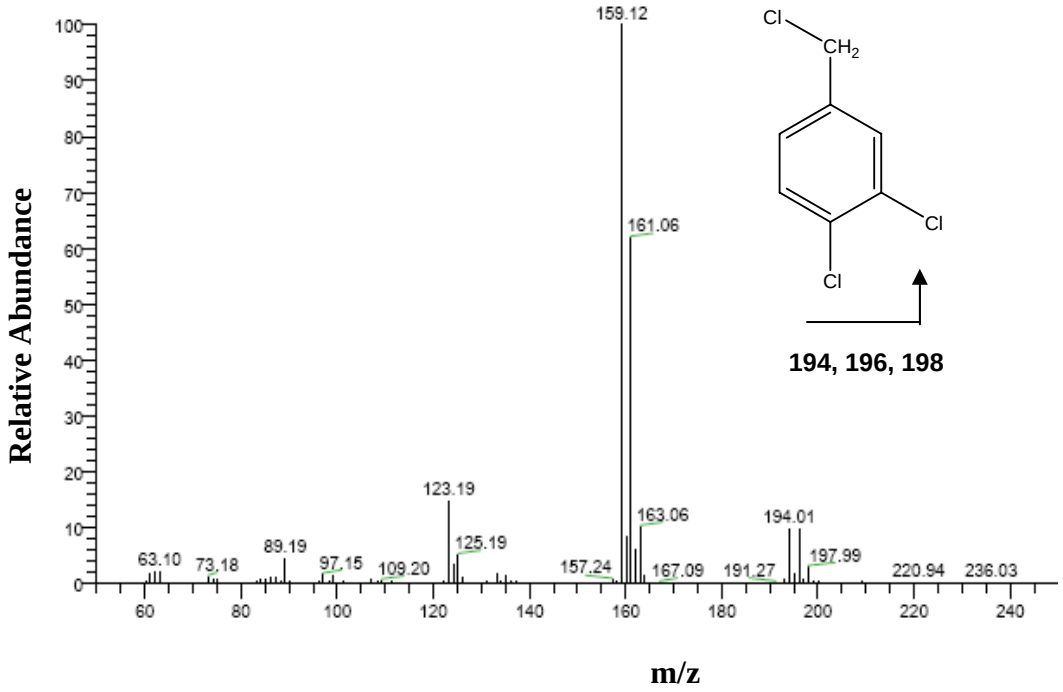

Figure 5.2c Full scan mass spectrum (*m/z* 50-250) of (B) 3,4,α-trichlorotoluene (istd)

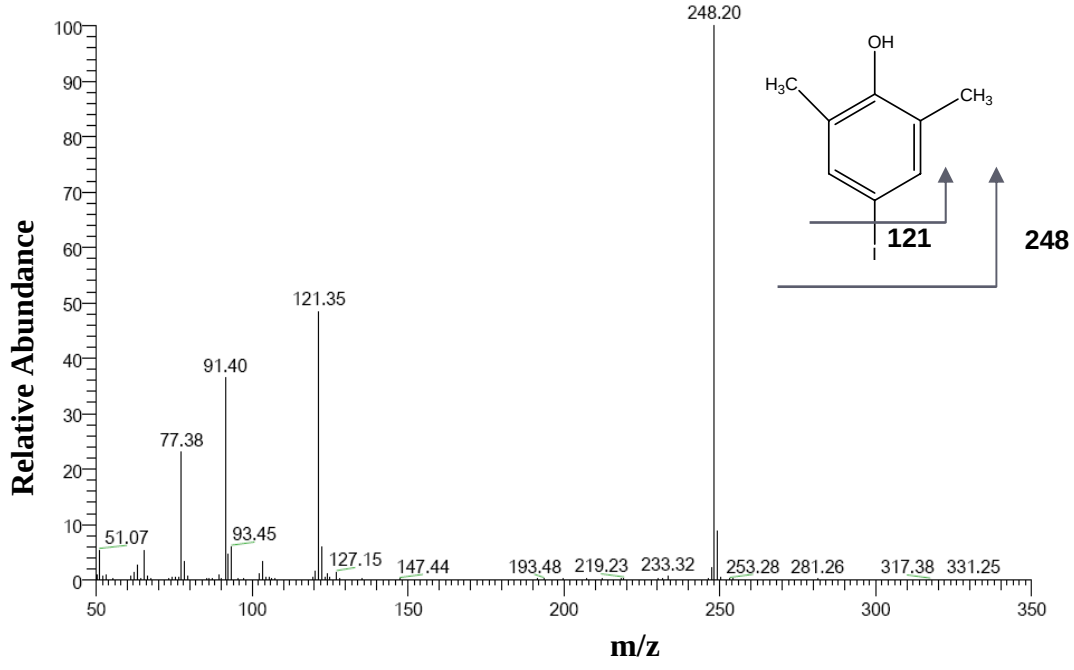

Figure 5.2d Full scan mass spectrum (*m/z* 50-350) of (C) 4-iodo-2,6-dimethylphenol (derivative)

5.3.2 Iodide Determination using 2,6-Dimethylphenol as Derivatizing Agent and 2,4,6-Trichlorophenol as Internal Standard

2,6-Dimethylphenol (excess derivatizing agent), 2,4,6-trichlorophenol (internal standard, istd) and 4-iodo-2,6-dimethylphenol (iodo-derivative) were detected at 5.93, 8.37 and 9.84 min, respectively. 2,6-Dimethylphenol and 4-iodo-2,6-dimethylphenol show the molecular ion as the base ions, *m/z* 122 and 248, respectively. The 2,4,6-trichlorophenol mass spectrum shows characteristic natural isotope ratio of chlorine at molecular ion *m/z* 196, 198 and 200, respectively. Total ion chromatograms and mass spectra are shown in Figure 5.3.

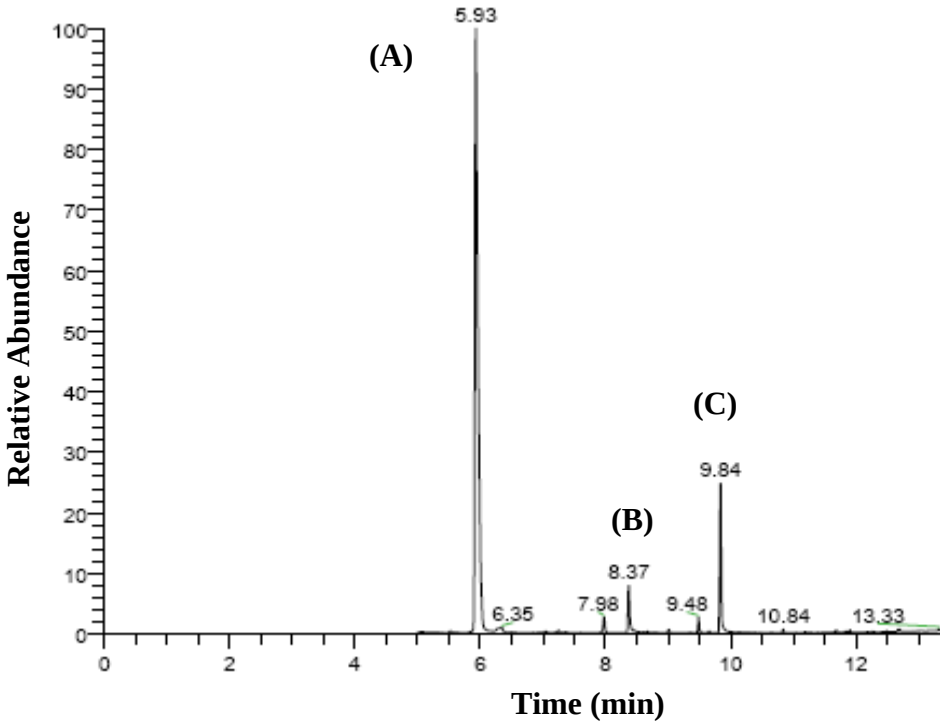

Figure 5.3a Total ion chromatogram (TIC) of the derivatives (1000 ppb iodide 3 mL, 1 mL MTBE extraction) : (A) 2,6-dimethylphenol (derivatizing agent); (B) 2,4,6-trichlorophenol (istd); (C) 4-iodo-2,6-dimethylphenol (derivative)

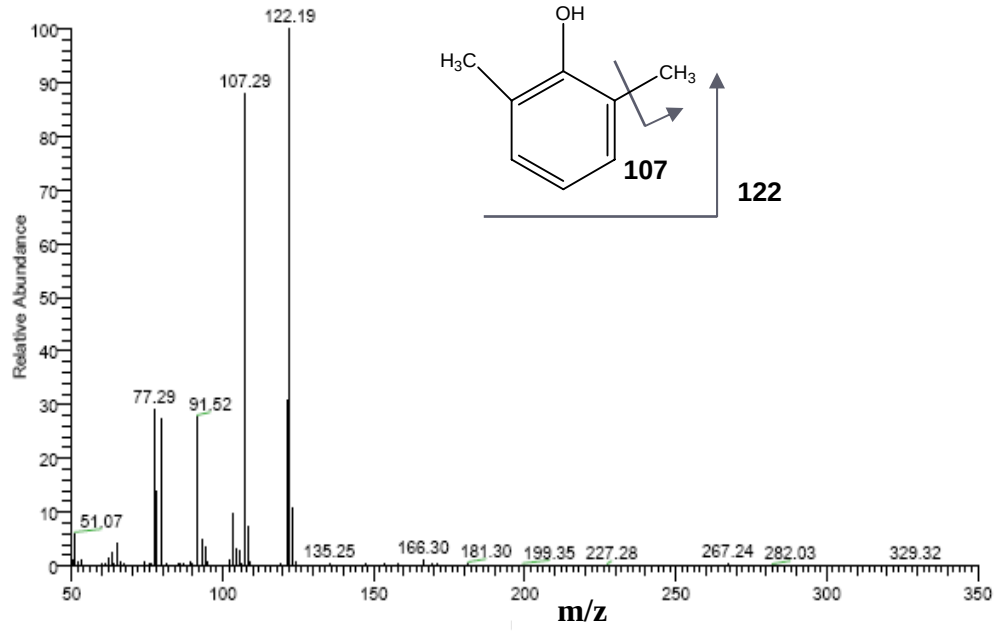

Figure 5.3b Full scan mass spectrum (*m/z* 50-350) of (A) 2,6-dimethylphenol (derivatizing agent)

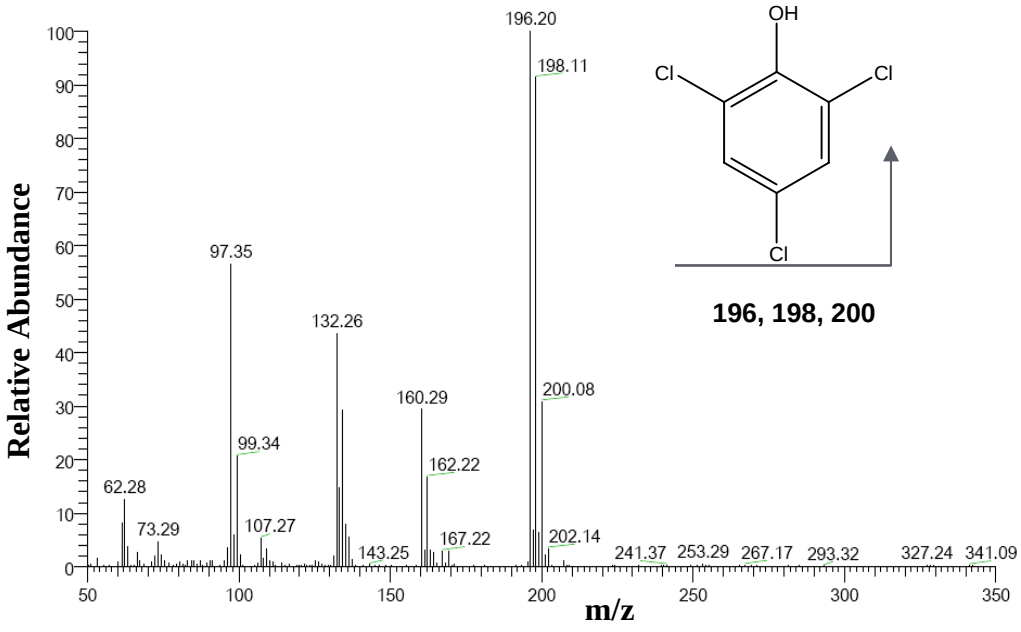

Figure 5.3c Full scan mass spectrum (*m/z* 50-350) of (B) 2,4,6-trichlorophenol (istd)

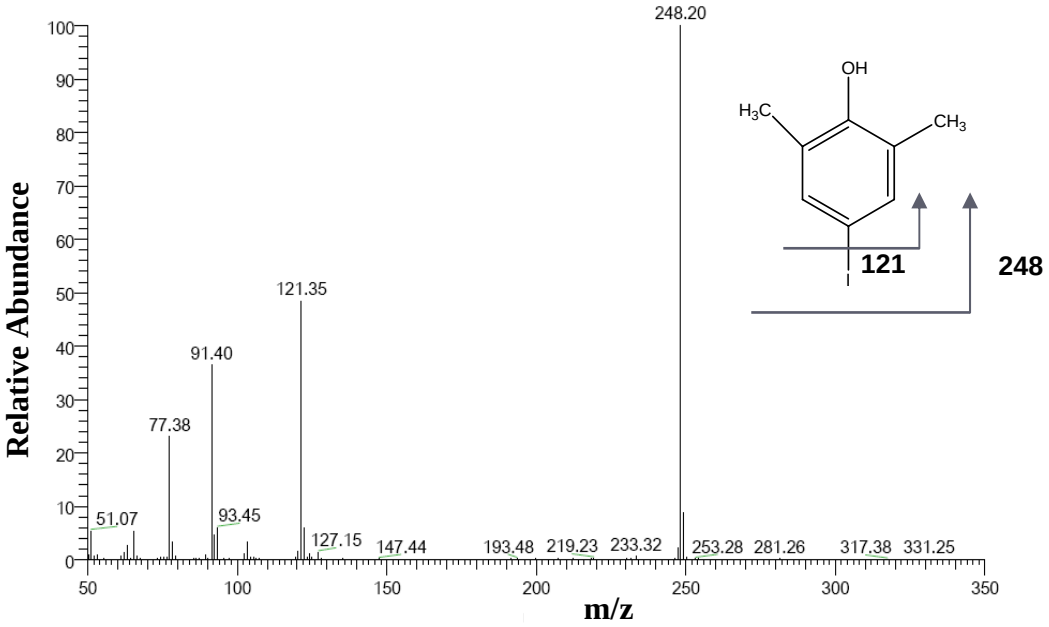

Figure 5.3d Full scan mass spectrum (*m/z* 50-350) of (C) 4-iodo-2,6-dimethylphenol (derivative)

5.3.3 Iodide Determination using *N,N*-dimethylaniline as Derivatizing Agent and Diphenylamine as Internal Standard

N,N-dimethylaniline (excess derivatizing agent) was eluted at 4.92 min but instrument was set to collect data after 6 min. 4-Iodo-*N,N*-dimethylanilene (iodo-derivative) and diphenylamine (istd) were detected at 7.51 and 8.02 min, respectively. 4-Iodo-*N,N*-dimethylaniline and diphenylamine show the molecular ion as the base ions, *m/z* 247 and 169, respectively. Total ion chromatograms and mass spectra are shown in Figure 5.4.

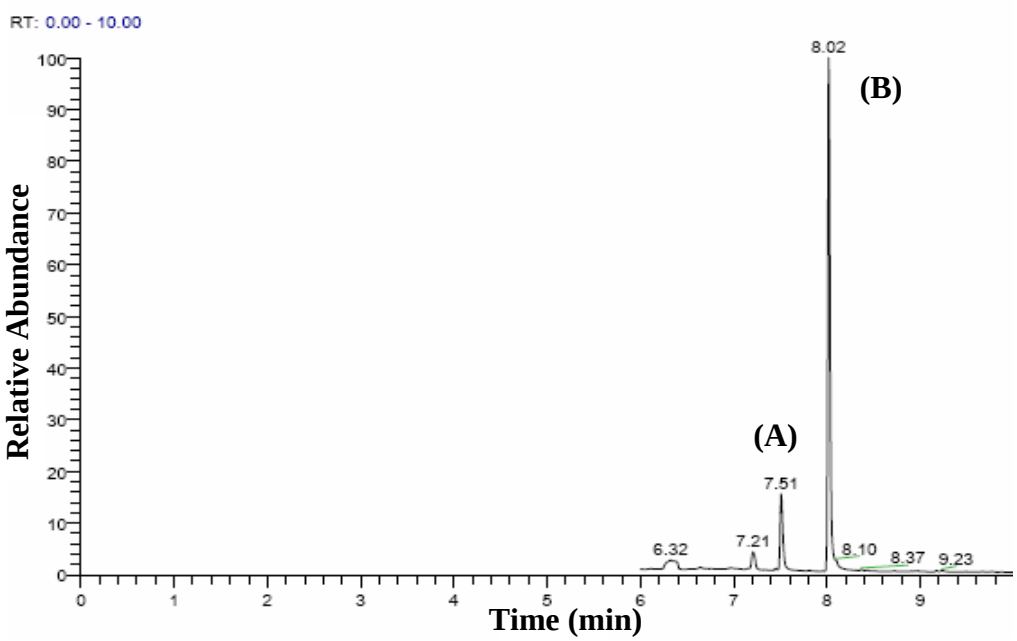

Figure 5.4a Total ion chromatogram (TIC) of the derivatives (200 ppb iodide 3 ml, 1 mL n-hexane extraction) : (A) 4-iodo-*N*,*N*-dimethylaniline (derivative) ; (B) diphenylamine (istd)

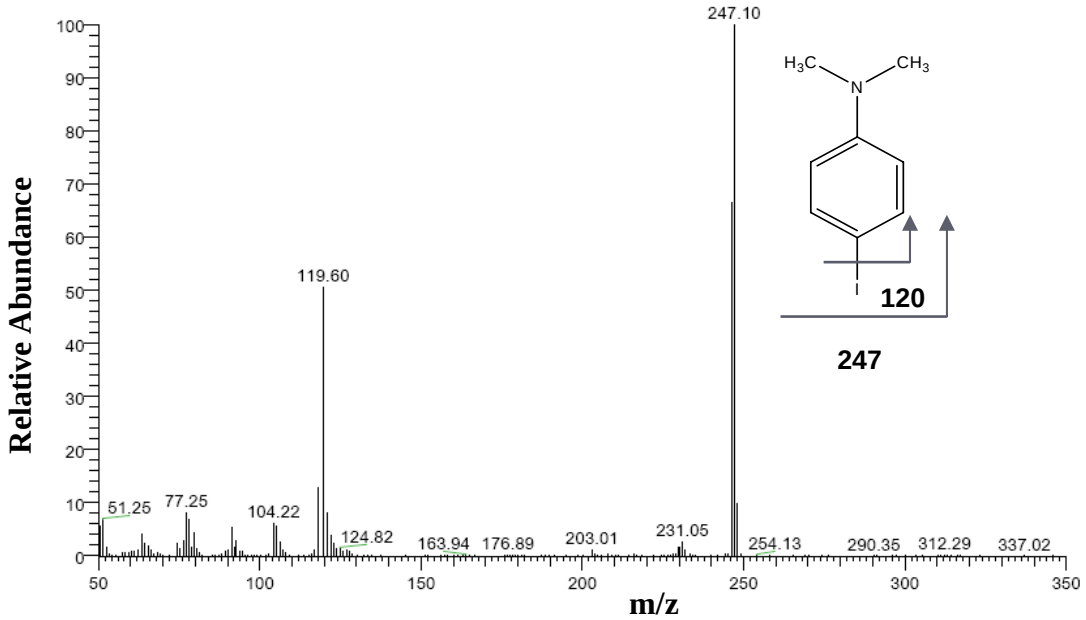

Figure 5.4b Full scan mass spectrum (*m/z* 50-350) of (A) 4-iodo-*N*,*N*-dimethylaniline (derivative)

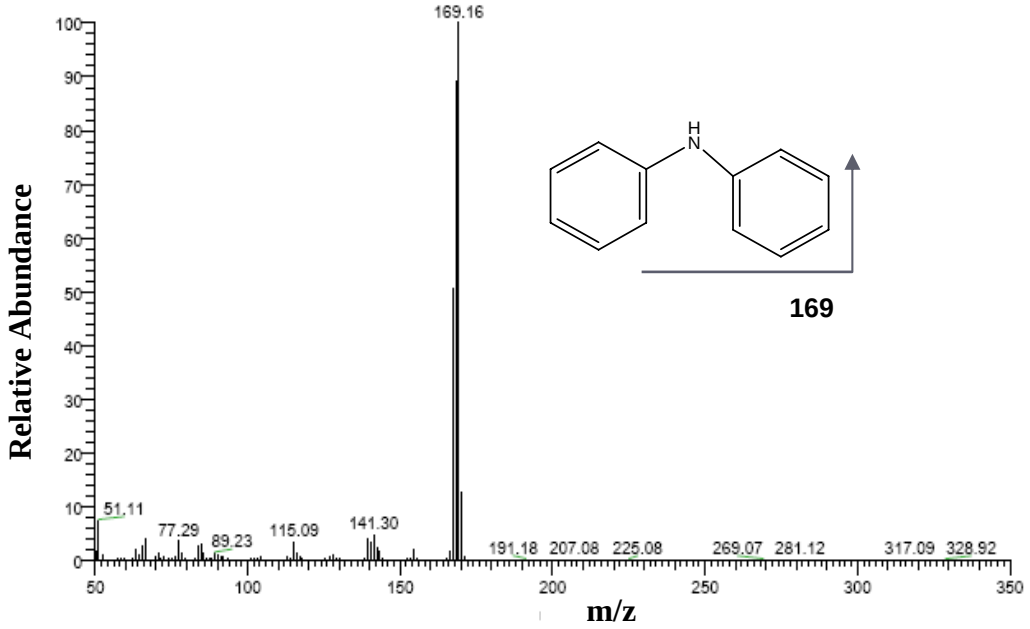

Figure 5.4c Full scan mass spectrum (*m/z* 50-350) of (B) diphenylamine (istd)

5.3.4 Iodide Determination using 2,6-Dimethylaniline as Derivatizing Agent and Diphenylamine as Internal Standard

2,6-dimethylaniline (excess derivatizing agent) was eluted at 6.84 min but instrument was set to collect data after 7 min. 4-Iodo-2,6-dimethylanilene (iodo-derivative) and diphenylamine (istd) were detected at 11.58 and 11.84 min, respectively. 4-Iodo-2,6-dimethylphenol shows the molecular ion as the base ion, *m/z* 247. Diphenylamine shows the molecular ion as the base ion, *m/z* 169. Total ion chromatograms and mass spectra are shown in Figure 5.5.

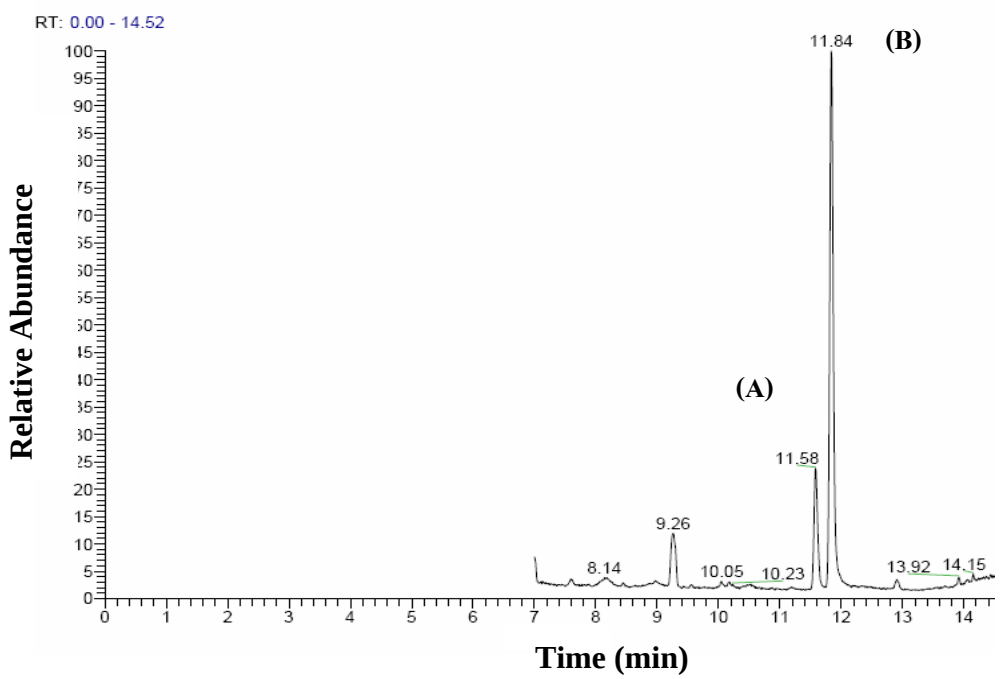

Figure 5.5a Total ion chromatogram (TIC) of the derivatives (250 ppb iodide 3 ml, 1 mL n-hexane extraction) : (A) 4-iodo-2,6-dimethylaniline (derivative) ; (B) Diphenylamine (istd)

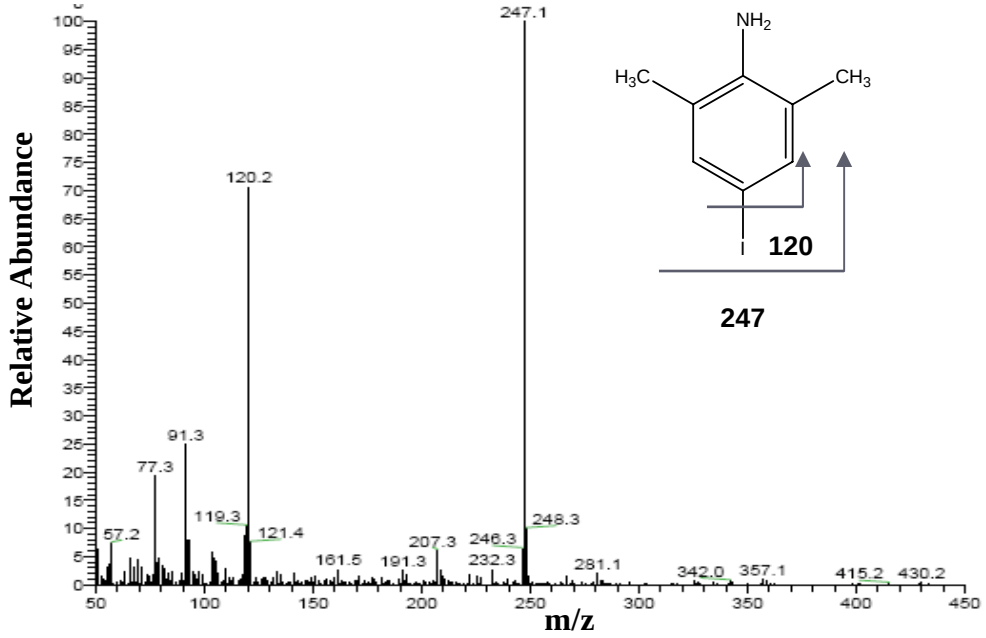

Figure 5.5b Full scan mass spectrum (*m/z* 50-450) of (A) 4-iodo-2,6-dimethylaniline (derivative)

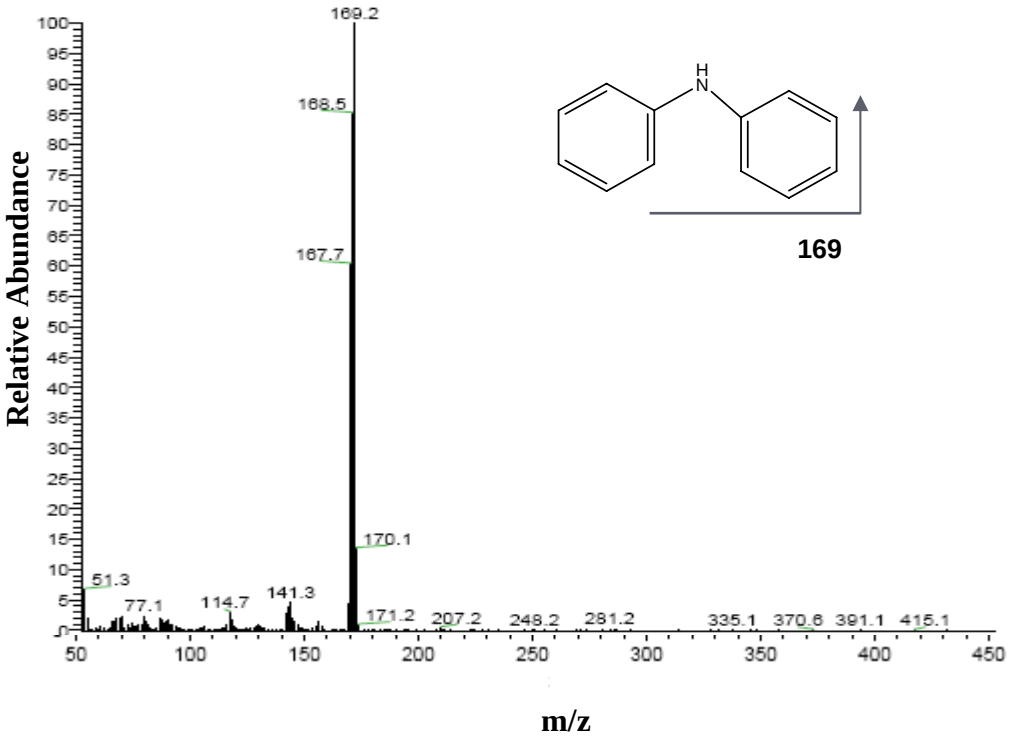

Figure 5.5c Full scan mass spectrum (*m/z* 50-450) of (B) Diphenylamine (istd)

5.3.5 Iodide Determination using 2,6-Diisopropylphenol as Derivatizing Agent and Diphenylamine as Internal Standard

2,6-Diisopropylaniline can derivatize iodine to 4-iodo-2,6-diisopropylaniline because of amine and isopropyl groups in the aromatic ring. The amine group is a strong activating substituent, classified as ortho- and para-direction but at ortho-position is substituted with isopropyl group, so only the para-position is available for iodine.

2,6-Diisopropylaniline (excess derivatizing agent) was eluted at 8.13 min. Diphenylamine (istd) and 4-iodo-2,6-diisopropylanilene (iodo-derivative) were detected at 9.94 and 12.79 min, respectively. 2,6-Diisopropylaniline, 4-iodo-2,6-diisopropylaniline and diphenylamine show the molecular ion *m/z* 178, 304 and 169, respectively. There was an unknown peak co-eluting with the iodo-derivative at RT 12.83. Total ion chromatograms and mass spectra are shown in Figure 5.6.

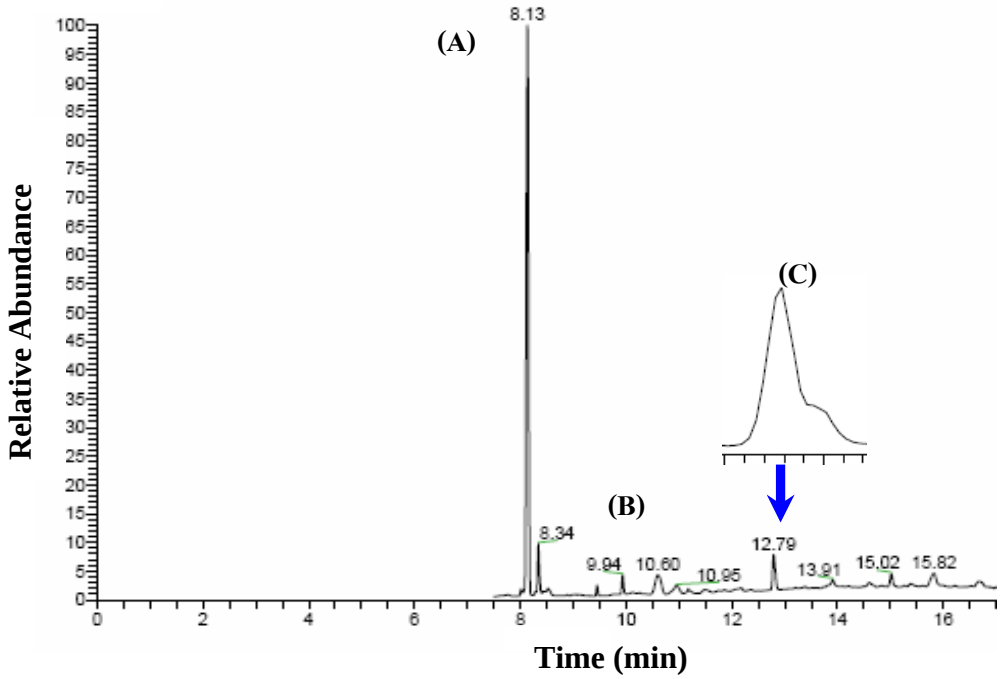

Figure 5.6a Total ion chromatogram (TIC) of the derivatives (250 ppb iodide 3 ml, 1 mL n-hexane extraction) : (A) 2,6-diisopropylaniline (derivatizing agent) ; (B) Diphenylamine (istd) ; (C) 4-iodo-2,6-diisopropylaniline (derivative)

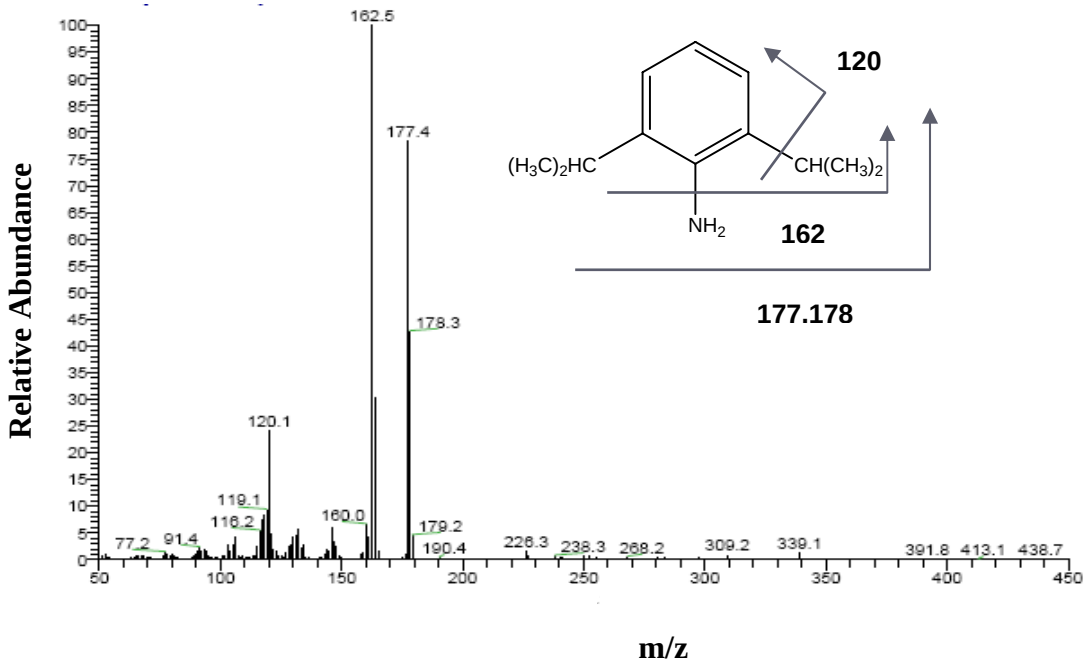

Figure 5.6b Full scan mass spectrum (*m/z* 50-450) of (A) 2,6-diisopropylaniline (derivatizing agent)

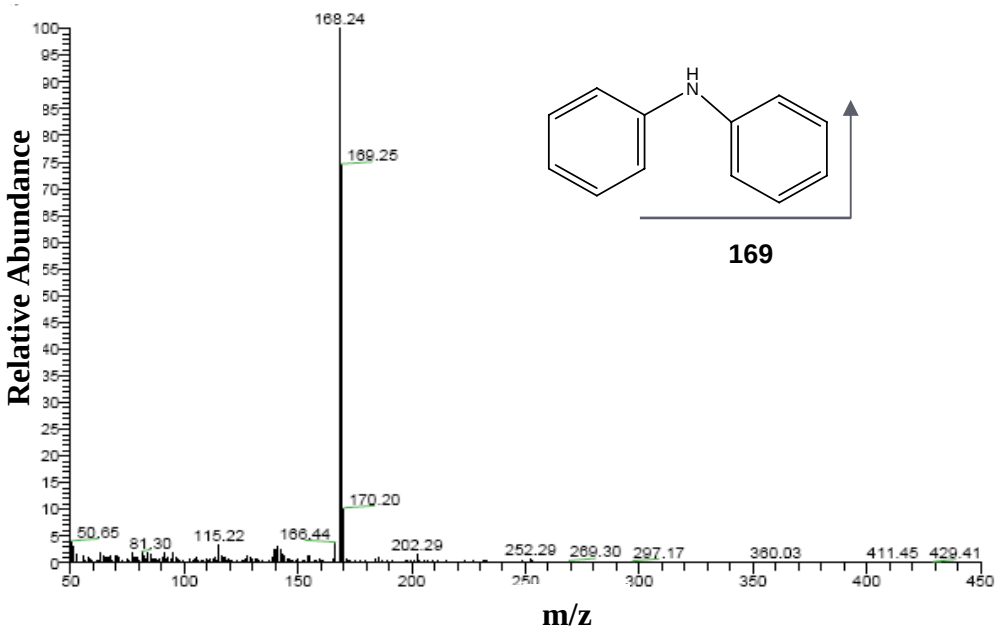

Figure 5.6c Full scan mass spectrum (*m/z* 50-450) of (B) diphenylamine (istd)

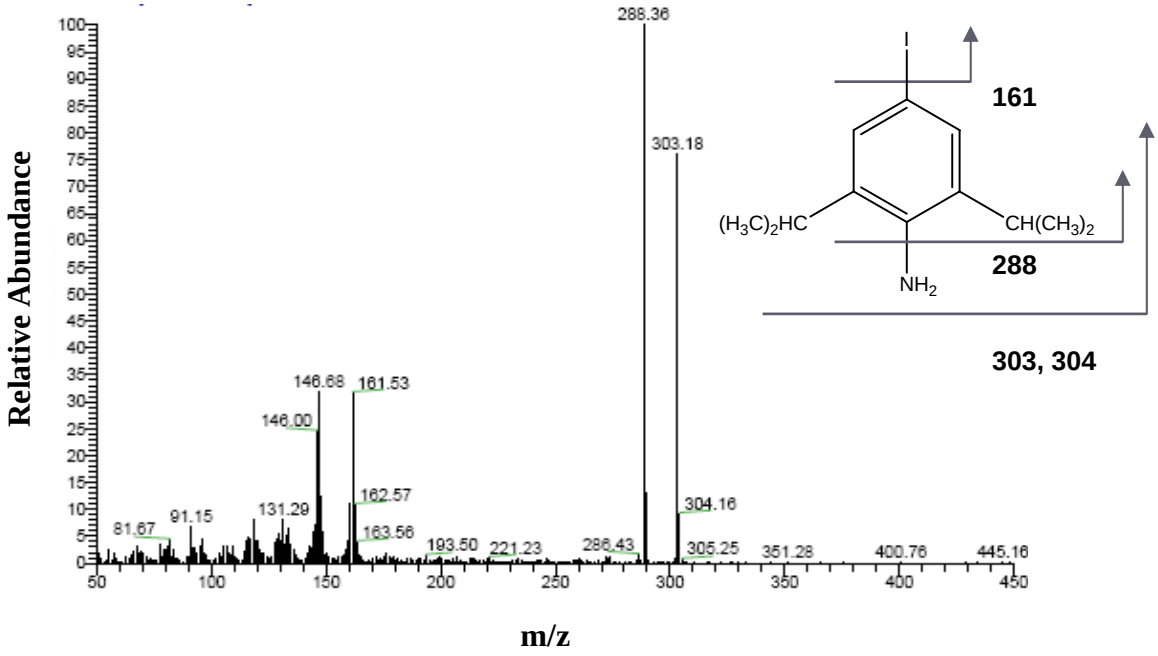

Figure 5.6d Full scan mass spectrum (*m/z* 50-450) of (C) 4-iodo-2,6-diisopropylaniline (derivative)

2,6-Diisopropylaniline is only 92% purity. Impurity peak eluted near derivatizing agent at 8.34 min. Characteristic mass spectrum is similar to 2,6-

diisopropylaniline (see Figure 5.6e). Impurity has the molecular ion *m/z* 178 and fragmentation of molecular ion by loss of methyl group m/z 162. From library search, the impurity is probably 2-sec-butyl-6-ethylaniline. Like 2,6-diisopropylaniline, 2-sec-butyl-6-ethylaniline can another derivatize iodine to 4-iodo-derivative.

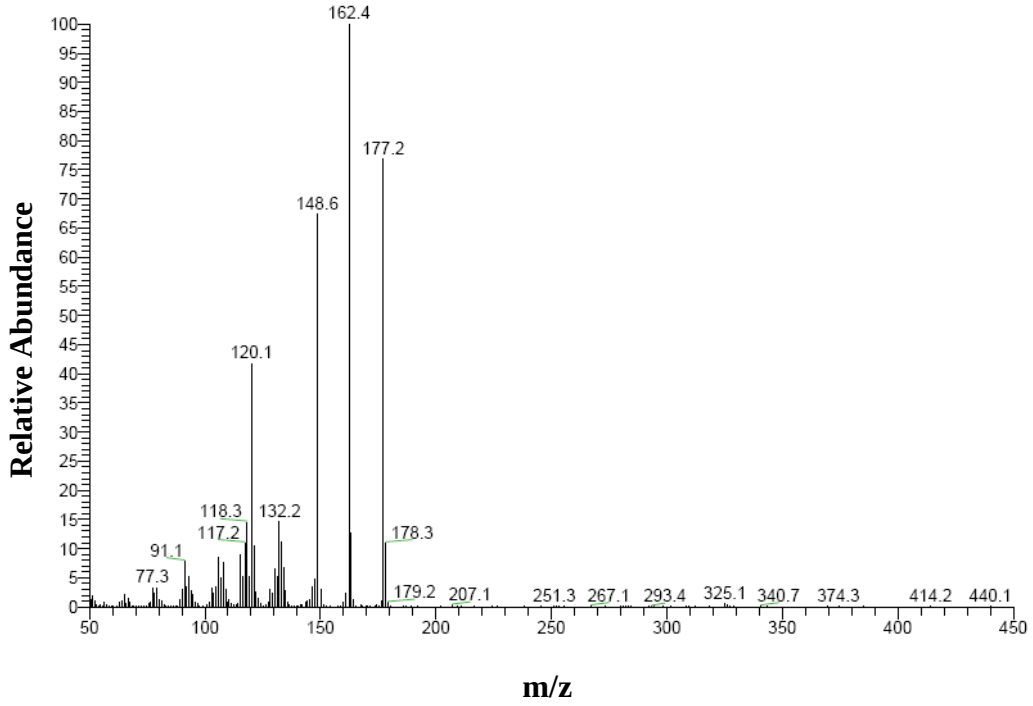

Figure 5.6e Full scan mass spectrum (*m/z* 50-450) of impurity at RT 8.34 min

Although, 4-iodo-2,6-diisopropylaniline can be separated and analysed by GC-MS, the impurity in the 2,6-diisopropylaniline (92% purity) may react with the iodine. And in the total ion chromatogram (Figure 5.6a), unknown peak co-eluted at 12.83 min close to the iodo-derivative. Thus, 2,6-diisopropylaniline was not further studied.

5.4 Internal Standard

Internal standard is a compound added to a sample in known concentration to facilitate the quantitative determination of the sample components [49]. Substance which is similar in the chemical behavior and analytical response to a certain target analyte should be chosen as internal standard. In this work, a fixed volume of the

internal standard solution is added to both the sample and the calibration solutions. When the sample and the calibration solutions are analysed, the peaks for both the internal standard and the target analyte are integrated. The area of the analyte peak is divided by the area of the internal standard peak to produce a peak area ratio value. This method corrects for run-to-run-variation in extraction efficiency, chromatographic response and ionization variability.

Before using 2,4,6-trichlorophenol as internal standard for 2,6-dimethylphenol derivatization, 3,4,α-trichlorotoluene was studied.

Previously, Mishra *et al.* [13], Reddy-Noone *et al.* [14] and Das *et al.* [46] proposed 2,4,6-tribromoaniline and 4-bromo-*N*,*N*-dimethylaniline as internal standard, respectively. In this work, diphenylamine was studied as internal standard for *N*,*N*-dimethylaniline and 2,6-dimethylaniline derivatization.

5.4.1 3,4,α-Trichlorotoluene as Internal Standard for 2,6-Dimethylphenol Derivatization

3,4,α-Trichlorotoluene is an aromatic hydro-carbon with three chloro- and one methyl functional groups. 3,4,α-Trichlorotoluene can separated from derivatizing agent and derivative and detected by GC-MS (see Figure 5.2a).

The linear calibration curve gave correlation coefficient (r^2) 0.9564. The correlation coefficient was lower than 0.99. Hence 3,4,α-trichlorotoluene was not appropriate as internal standard for 2,6-dimethylphenol derivatization for 10-250 ppb of iodide concentration. Standard curve is shown below.

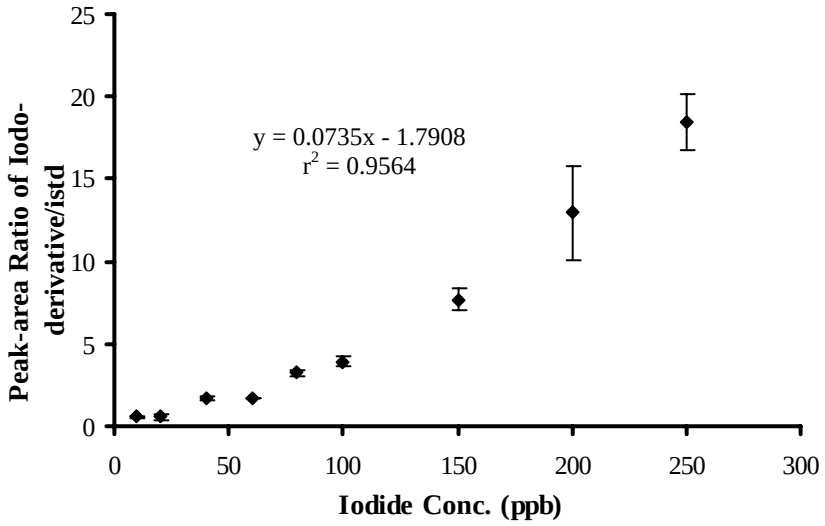

Figure 5.7 Standard curve of iodo-2,6-dimethylphenol in concentration range 10-250 ppb of 3 mL iodide standard solution with 3,4,α-trichlorotoluene as internal standard

However, for 150-250 ppb range, correlation coefficient is 0.9999 (see Appendix C for calibration line).

5.4.2 2,4,6-Trichlorophenol for 2,6-Dimethylphenol Derivatization

2,4,6-Trichlorophenol is a phenol compound with chloro substituent at the ortho- and para- positions. 2,4,6-Trichlorophenol is similar to 4-iodo-2,6-dimethylphenol in structure and polarity.

Correlation coefficient for 10-250 ppb concentration range of iodide using 2,4,6-trichlorophenol as internal standard was more than 0.99 (see Figure 5.12). 2,4,6-Trichlorophenol was thus a suitable internal standard for 2,6-dimethylphenol derivatization.

5.4.3 Diphenylamine as Internal Standard for *N,N*-dimethylaniline and 2,6-Dimethylaniline Derivatization

Diphenylamine is an aniline compound which is nearby available for *N*,*N*-dimethylaniline and 2,6-diemthylaniline. Thus, diphenylamine was studied as internal standard for *N*,*N*-dimethylaniline and 2,6-dimethylaniline derivatization.

Correlation coefficients are more than 0.99 for *N*,*N*-dimethylaniline and 2,6-dimethylaniline derivatization in 10-250 ppb concentration range of iodide (see Figure 5.13 and 5.14, respectively). Diphenylamine was thus selected as an internal standard for *N*,*N*-dimethylaniline and 2,6-dimethylaniline derivatization in iodide concentration range 10-250 ppb.

5.5 Time Period for Derivatization

5.5.1 Preliminary Data

Using Mishra *et al*'s procedure, the curve of area ratio of derivative to internal standard with time was not constant even after 600 min. The results are shown in Figure 5.8

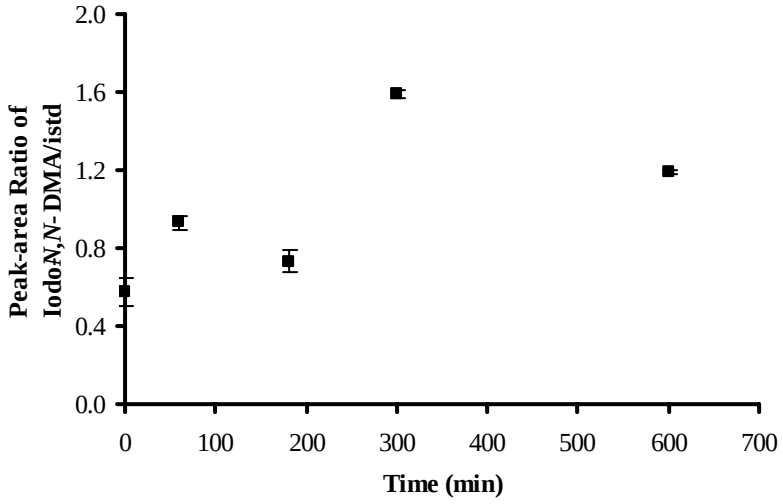

Figure 5.8 Result of derivatization of 750 ng iodide (250 ppb of 3 mL iodide standard solution) with *N*,*N*-dimethylaniline in 25 mL aqueous solution and extraction with 200 μL n-hexane at various time. Diphenylamine was used as the internal standard.

The volume of the extraction solvent, hexane was then increased to 400 μL. The curve of area ratio of derivative to internal standard with time was constant after 150 min. The results are shown in Figure 5.9.

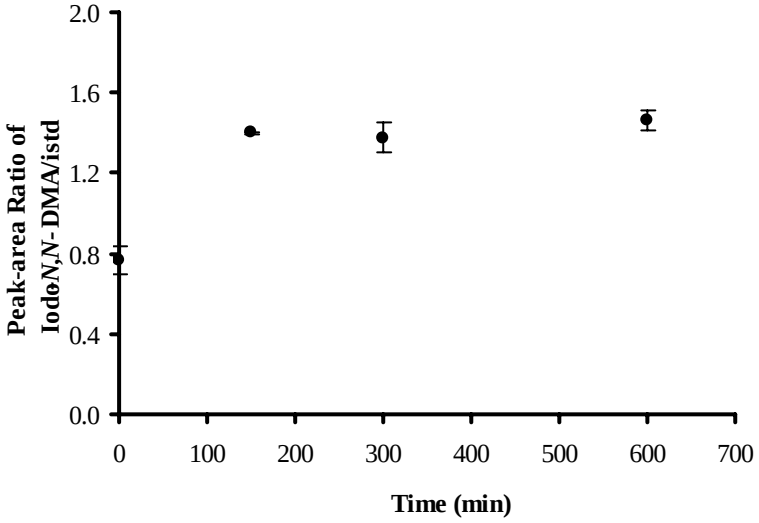

Figure 5.9 Result of derivatization of 750 ng iodide (250 ppb of 3 mL iodide standard solution) with *N,N*-dimethylaniline in 25 mL aqueous solution and extraction with 400 μL n-hexane versus time. Diphenylamine was used as the internal standard.

A 1.00 mL aliquot of n-hexane was next employed. The procedure followed as described in Section 4.5.1b). The curve of area ratio of derivative to internal standard with time was constant after 1 min reaction time. The results are shown in Figure 5.10.

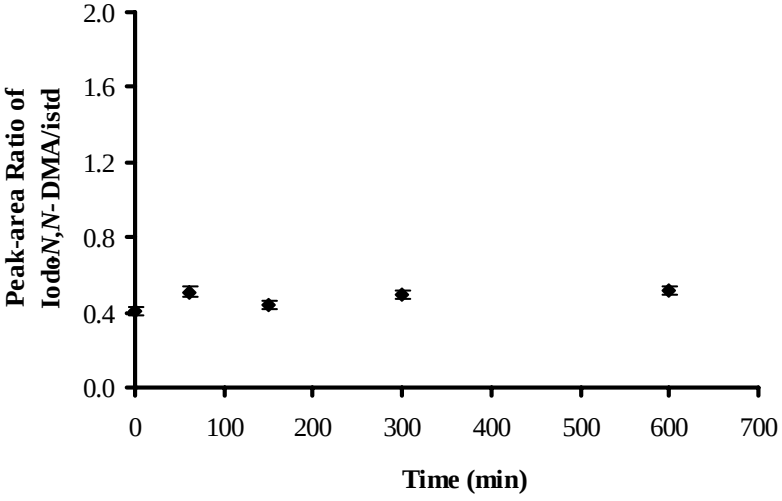

Figure 5.10 Result of derivatization of 250 ppb of 3 mL iodide standard solution with *N*,*N*-dimethylaniline in 4.5 mL aqueous solution and extraction with 1 mL n-hexane versus time. Diphenylamine was used as the internal standard.

Thus, the reaction time complete within 1 min, but suitable volume of extract solvent required large extract volume.

5.5.2 Time Period for Derivatization Study

Shin *et al.* [12], Mishra *et al.* [13] and Reddy-Noone *et al.* [14] reported reaction times of iodide derivatization by 2,6-dimethylphenol, *N*,*N*-dimethylaniline and 2,6-dimethylaniline, respectively. There were 20, 1 and 10 minute, respectively. The reaction rates of iodide with derivatizing agents were confirmed using a 1 mL organic solvent extraction.

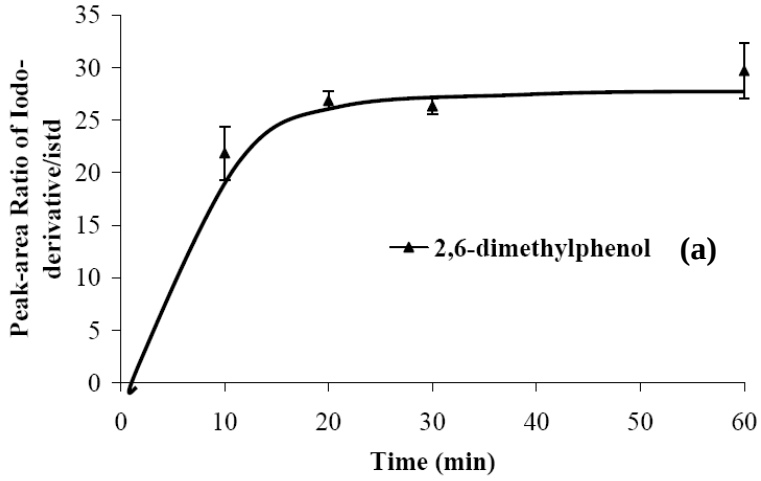

Figure 5.11a Reaction rate of iodide (250 ppb, 3 mL) derivatization with 2,6-dimethylphenol.

Peak area ratio of iodo-2,6-dimethylphenol to internal standard gave reproducible result for reaction times after 20 min, so complete reaction of iodo-2,6-dimethylphenol derivatization took place in about 20 min at room temperature.

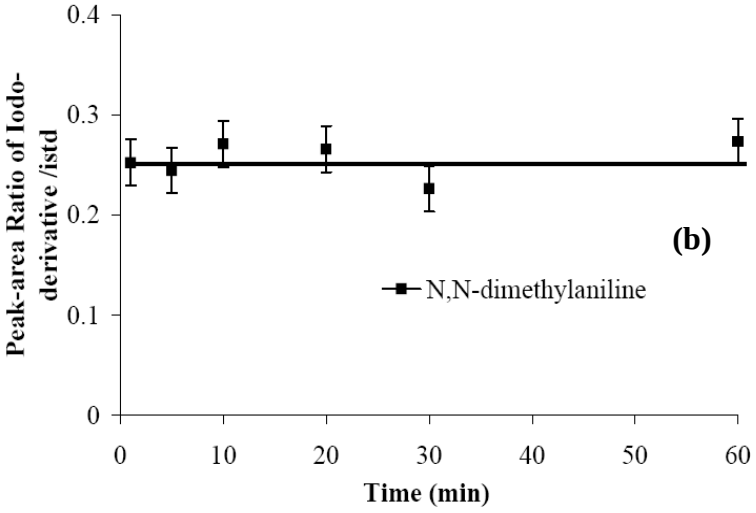

Figure 5.11b Reaction rate of iodide (250 ppb, 3 mL) derivatization with *N*,*N*-dimethylaniline.

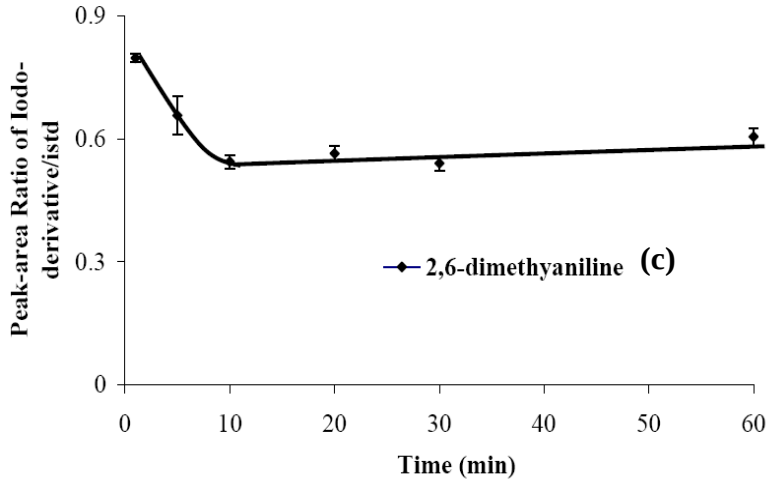

Figure 5.11c Reaction rate of iodide (250 ppb, 3 mL) derivatization with 2,6-dimethylaniline.

Complete reaction of *N*,*N*-dimethylaniline derivatization was within 1 min at room temperature because peak area ratio of iodo-*N*,*N*-dimethylaniline to internal standard gave reproducible result with reaction time from 1-60 min. 4-Iodo-2,6-dimethylaniline reaction was complete in about 10 min at room temperature.

5.6 Analytical Performance

5.6.1 Working Range and Linearity

The working range was 10-250 ppb of 3 mL iodide standard solution. The calibration graphs are the peak-area ratios of iodo-derivative to the internal standard for this concentration range. The criterion for acceptable linearity is correlation coefficient (r^2) higher than 0.995.

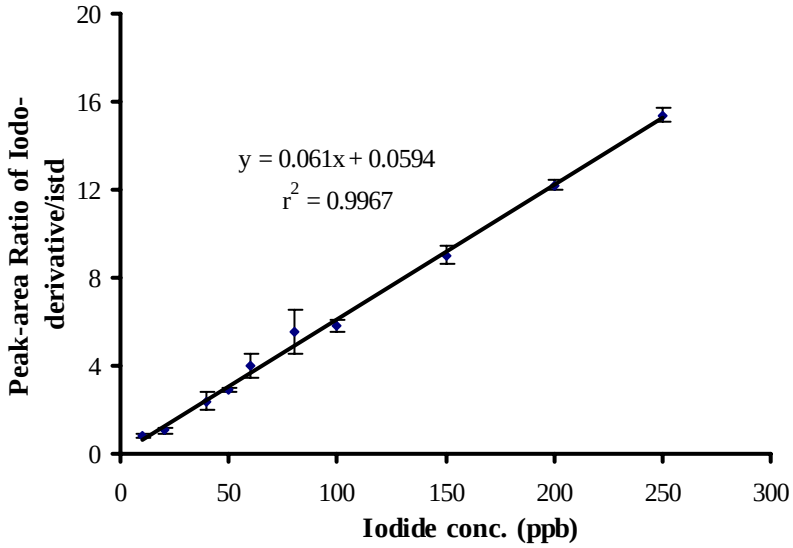

Figure 5.12 Calibration curve of 4-iodo-2,6-dimethylphenol

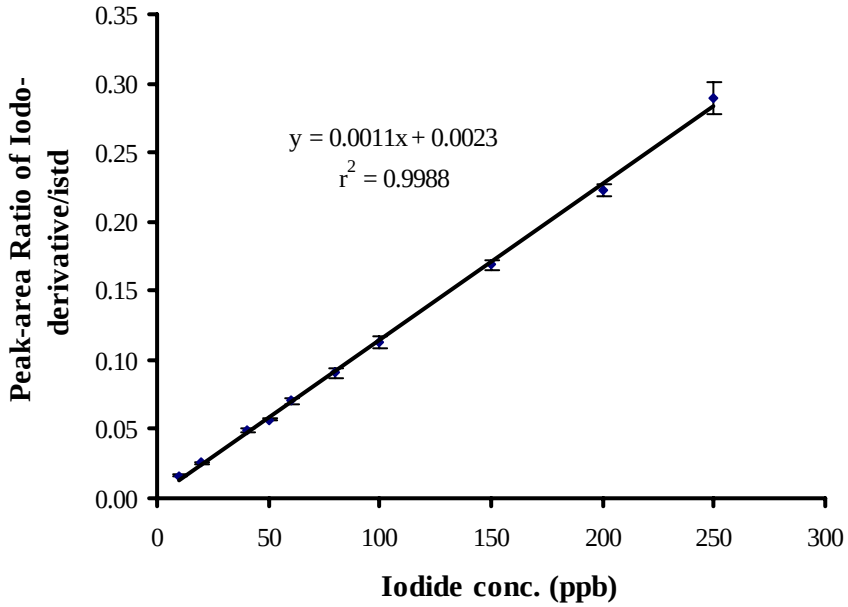

Figure 5.13 Calibration curve of 4-iodo-*N*,*N*-dimethylaniline

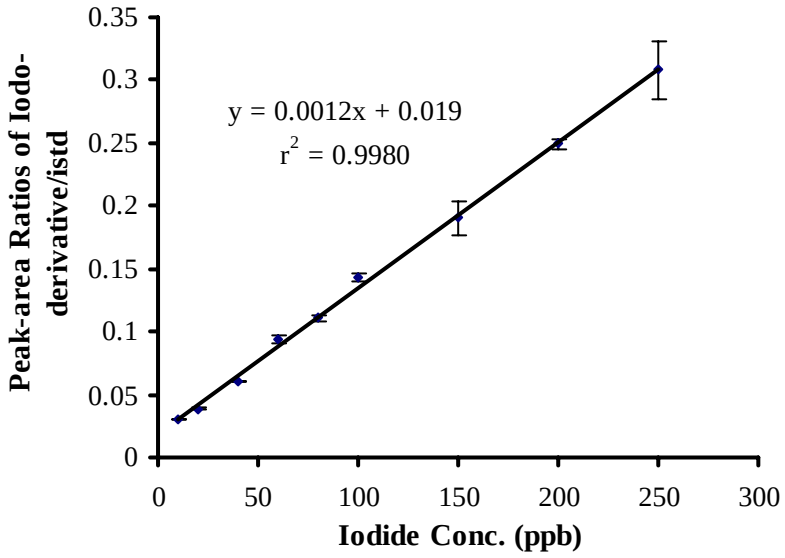

Figure 5.14 Calibration curve of 4-iodo-2,6-dimethylaniline

4-Iodo-2,6-dimethylphenol, 4-iodo-*N*,*N*-dimethylaniline and 4-iodo-2,6-dimethylaniline gave good linearity for iodide concentration 10-250 ppb (see Figure 5.12-5.14).

Table 5.1 Working range and linearity of derivative

Derivative	Working Range of Iodide (ppb)	Equation	Correlation Coefficient (r^2)
4-iodo-2,6-dimethylphenol	10-250	y = 0.061(±0.001)x + 0.059(±0.152)	0.9967
4-iodo-*N*,*N*-dimethylaniline	10-250	y = 0.0011(±0.0001)x + 0.0023(±0.0016)	0.9989
4-iodo-2,6-dimethylaniline	10-250	y = 0.00116(±0.00002)x + 0.01898(±0.00248)	0.9980

5.6.2 Limit of Detection

Detection limit was calculated from the average area ratios of 6 blanks plus three standard deviations. They were 6.42, 3.71 and 3.35 ppb for iodo-2,6-dimethylphenol, iodo-*N*,*N*-dimethylaniline and iodo-2,6-dimethylaniline, respectively. The ion chromatogram of reagent blank and iodide at limit of detection are shown below.

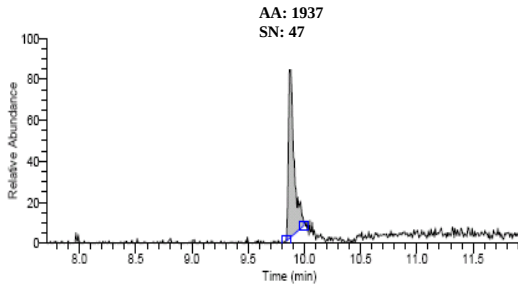

Figure 5.15a Ion chromatogram and peak area of *m/z* 248 of reagent blank; The area ratio to istd is 0.4170.

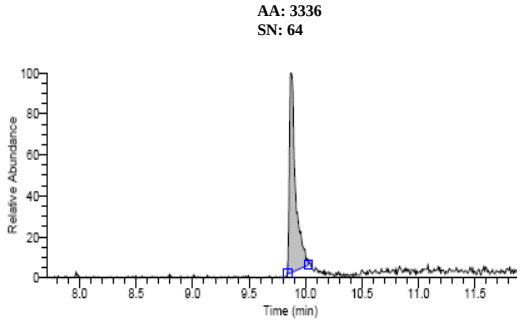

Figure 5.15b Ion chromatogram and peak area of *m/z* 248 of 4-iodo-2,6-dimethylphenol for 6.42 ppb of 3 mL iodide standard solution; The area ratio to istd is 0.6962.

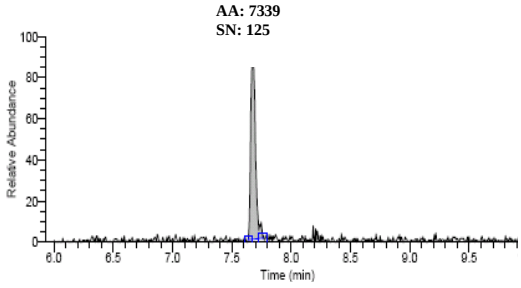

Figure 5.16a Ion chromatogram and peak area of *m/z* 247 of reagent blank; The area ratio of derivative to istd is 0.0112.

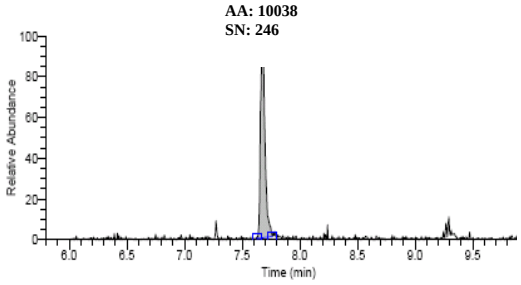

Figure 5.16b Ion chromatogram and peak area of *m/z* 247 of 4-iodo-*N*,*N*-dimethylphenol for 3.71 ppb iodide standard solution; The area ratio to istd is 0.0146.

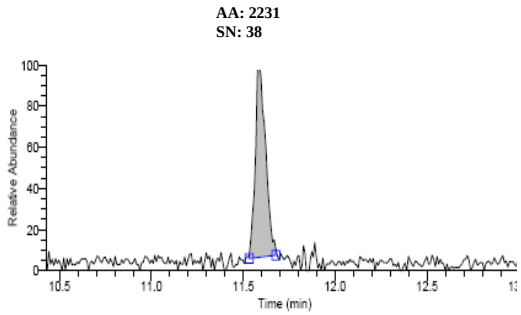

Figure 5.17a Ion chromatogram and peak area of *m/z* 247 of reagent blank; The area ratio of derivative to istd is 0.0070.

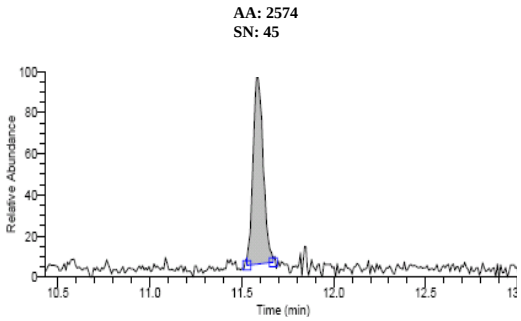

Figure 5.17b Ion chromatogram and peak area of *m/z* 247 of 4-iodo-2,6-dimethylaniline for 3.35 ppb iodide standard solution; The area ratio to istd is 0.0097.

From the results of linearity and detection limit, the aniline derivatives gave better correlation coefficient and detection limit than the iodo-phenol compound. The correlation coefficient and detection limit for 4-iodo-*N*,*N*-dimethylaniline was comparable with 4-iodo-2,6-dimethylaniline.

N,*N*-dimethylaniline derivatization gave the shortest derivatization time consuming (see Section 5.5). The *N*,*N*-dimethylaniline was chosen as the derivatizing agent for further study.

5.6.3 Accuracy and Precision

The accuracy of the method was tested by analyzing a 1000 μg/mL iodide certified standard solution which is traceable to National Institute of Standard and Technology (NIST). Analytical values were compared with the certified value.

Precision was calculated from six certified standard solutions. The criterion is less than 5 relative standard deviation percentile which predicted RSD for repeatability calculating from Horwitz equation. Analytical value is 966.0 μg/mL. Relative standard deviation percentile is 4.25.

Table 5.2 Result of accuracy and precision

Items	**Accuracy**	**Precision**
Criteria	$t_{observed} < t_{critical}$	$\leq$ 5%RSD
Analytical value	2.03[a]	4.25[c]
Criteria value	2.57[b]	5[c]
Result	2.03<2.57	4.25$\leq$5

[a] $t_{observed,\ n=6}$

[b] $t_{critical,\ n=6}$ two tail at 95% confidence

[c] %RSD

The method for as iodide determination using *N*,*N*-dimethylaniline as the derivatizing agent by GC-MS was found to be accurate and precise.

5.7 Sample Cleanup by Solid Phase Extraction (SPE)

Instant noodle seasonings consist of many materials which can interfere with the quantitative conversion of iodine to iodo-derivative. Cleanup method was studied for interference elimination. Solid phase extraction (SPE) techniques have been developed to replace many traditional extraction methods. SPE methods are easier to perform and provide a means to process samples quickly, less solvent consumption and more reproducible results.

Sep-Pak (C18) which is usually used for extracting organic compound and strong anion exchange (SAX) were chosen for recovery study.

5.7.1 Recovery Study

The recovery study optimized suitable condition of the SPE for iodide recovery of about 100%. The iodide standard solution was at the concentration of iodide that is expected in sample solution. Then, the optimal extraction conditions were applied to sample.

Table 5.3 Recovery study of SPE in aqueous solution

SPE Type / **I in Aqueous.**	**Sep-Pak (C18)**	**Strong Anion Exchange (SAX)**
%Recovery ±SD	93.54±0.09(n=3)	92±3(n=2)
Wash/Elute volume	3 mL of water	10 mL of 3 M $NaNO_3$
%Recovery ±SD	99(n=1)	117(n=1)
Wash/Elute volume	4 mL of water	15 mL of 3 M $NaNO_3$

Sep-Pak gave 99% iodide recovery for 4 mL water washing and 117% iodide recovery for 15 mL of 3 M $NaNO_3$ elution in SAX. The optimum conditions are 4 mL water washing and 15 mL of 3 M $NaNO_3$ elution for Sep-Pak and SAX, respectively.

Table 5.4 Recovery study of SPE in sample

SPE Type / Sample	**Sep-Pak, C18 %Recovery Mean ± SD ; (n=3)**	**Strong Anion Exchange (SAX) %Recovery Mean ± SD; (n=3)**
Urine A	15 ± 8	36 ± 10
Urine B	22 ± 12	50 ± 8
Urine C	7 ± 6	12 ± 5
Instant Noodle A	63 ± 2	100 ± 3
Instant Noodle B	76 ± 7	101 ± 9
Instant Noodle C	82 ± 9	97 ± 2

The SPE methods with C18 and SAX were applied to urine samples. However, the results obtaining from both sorbents shown that some matrix in urine cannot be removed and interfered the derivatization reaction. The recoveries were lower than 50%. Therefore, extraction method, other sorbent materials and eluents were examined for eliminating the interference in urine such as mixed mode SPEs (MCX and MAX) and polymeric SPE including the use of SPE cartridges in series. The results showed that SPE with/without liquid-liquid extraction cannot remove some matrix in urine. The iodide content in urine cannot accurately determine using those sample preparations for GC-MS method.

On the other hands, mean recovery of iodide from Sep-Pak in 3 instant noodle seasonings of various flavors is 63–82%. Sep-Pak is therefore not suitable as a cleanup method for the seasoning samples.

For SAX, mean recovery of iodide were in the range of 97 - 101% . SAX gave better recovery than Sep-Pak in seasonings. Thus, SAX was used for sample cleanup.

5.7.2 Sample Application

Various samples were then applied using the clean up method with SAX. The results are shown in table 5.5.

5.8 Seasoning Sample Application

A value of iodide analyzed by GC-MS using external calibration, SAX cleanup and standard addition were compared with two methods that have been employed for seasoning namely ICP-MS and Sveikina method.

Table 5.5 Iodide content in seasoning by five different methods

Sample	Iodide Content in a Seasoning Sachet Mean ± SD (μg I)				
	GC-MS using External Calibration (n=1)	**GC-MS using SAX Cleanup (n=3)**	**GC-MS using Standard Addition (n=3)**	**ICP-MS Method (n=3)**	**Colorimetry based on Sveikina Method (n=3)**
A	36.8	ND	65.0 ± 4.2	71.30 ± 5.1	54.5 ± 3.5
B	ND	-[a]	4.1 ± 0.7	<12.50[a]	1.93 ± 0.9
C	ND	-[a]	5.8 ± 0.2	<12.50[a]	1.91 ± 0.7
D	ND	-[a]	4.5 ± 1.3	<12.50[a]	1.44 ± 0.6
E	8.55	ND	35.0 ± 0.9	33.47 ± 2.5	24.5 ± 2.6

[a] lower than working range

External calibration by GC-MS determined iodide in seasoning gave relatively lower than other method because of interferences. In eluent portion with 3 M $NaNO_3$ of cleanup method with SAX, the 4-iodo-*N*,*N*-dimethylaniline was not found by GC-MS technique. The derivatization was interfered with high concentration of nitrate. Mishra *et al*. reported the error of derivatization more than 1000-fold of nitrate [13].

Results of iodide determination using standard addition method by GC-MS are shown in table 5.5. They are 65.0, 4.1, 5.8, 4.50 and 35.0 μg for sample A, B, C, D and E, respectively.

ICP-MS can only detect iodide in seasoning greater than 12.5 μg because of high salt content in seasoning. These are 71.30 and 33.47 μg iodide in a sachet of seasoning sample A and E, respectively. However, the iodide content using ICP-MS and GC-MS with standard addition method of sample A and E were proved by statistical *t*-test. There are not significantly different at 95% confidence.

Colorimetry based on Sveikina method gave iodide content lower than ICP-MS and GC-MS using standard addition method (see table 5.5). The colorimetry based on Sveikina method was confirmed using iodide standard solution. The recoveries were 62-79% at 0.25-7.50 μg iodide in 50 mL aqueous solution (see in Appendix G). Iodide can be loss in ashing step [8]. Sample preparation by ashing should be avoided.

Sample preparation in fortified sample proposing by Perring *et al.* was dissolution of iodide in water and filtration by 0.2 μm membrane filter [46]. So, the sample portion filtering through 0.2 μm membrane filter was used for the colorimetry based on Sveikina method.

The result of iodide determination by GC-MS compared with ICP-MS and colorimetry method is shown in Figure 5.18

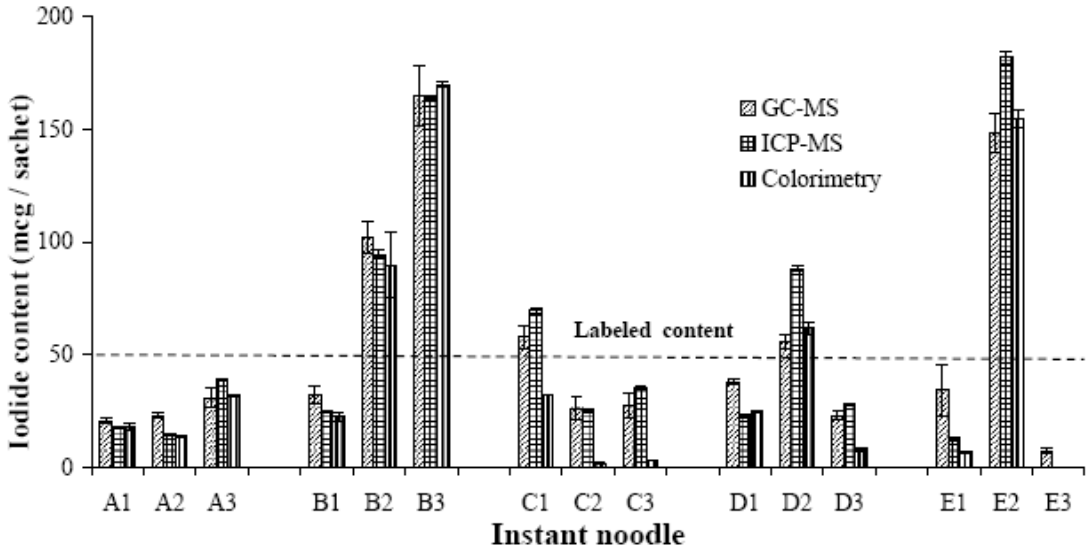

Figure 5.18 Iodide content in a sachet of instant noodle seasoning by GC-MS, ICP-MS and colorimetry

The iodide content of instant noodle seasoning determined by GC-MS and ICP-MS are not significantly different at 95% confidence. GC-MS and colorimetry are not significantly different at 95% confidence for sample A, B, D and E.

CHAPTER 6
CONCLUSION

Four derivatizing agents, namely 2,6-dimethylphenol, *N*,*N*-dimethylaniline, 2,6-dimethylaniline and 2,6-diiosopropylaniline, have been used for iodide determination by GC-MS after oxidation with 2-iodosobenzoate and derivatization with the derivatizing agent. 4-Iodo-2,6-dimethylphenol, 4-iodo-*N*,*N*-dimethylaniline, 4-iodo-2,6-dimethylaniline and 4-iodo-2,6-diiosopropylaniline were detected by MS. 4-Iodo-2,6-dimethylphenol, 4-iodo-*N*,*N*-dimethylaniline and 4-iodo-2,6-dimethylaniline gave clean separate peaks. The total ion chromatogram for derivatization with 2,6-diisopropylaniline of 92% purity gave co-eluting peak with 4-iodo-2,6-diisopropylaniline.

An internal standard of 2,6-dimethylphenol was 2,4,6-trichlorophenol. Diphenylamine was an internal standard for *N*,*N*-dimethylaniline and 2,6-dimethylaniline. The comparison of the three good derivatizing agents employed 1 mL organic solvent extract, 1 μL injection into GC-MS in splitless mode with a working range of 10-250 ppb of 3 mL iodide standard solution. The correlation coefficients (r^2) of the linear calibration curve plot peak-area ratio of iodo-derivative to internal standard relate to iodide concentration in 10-250 ppb were 0.9967, 0.9988 and 0.9980 for 4-iodo-2,6-dimethylphenol, 4-iodo-*N*,*N*-dimethylaniline and 4-iodo-2,6-dimethylaniline, respectively. The detection limits (blank + 3SD, n=6) were 6.42, 3.71 and 3.35 ppb, respectively.

The reaction was complete in 20 min for derivatization by 2,6-dimethylphenol, 1 min for derivatization by *N*,*N*-dimethylaniline and 10 min for 2,6-dimethylaniline was confirmed by GC-MS. Due to its rapid derivatizing agent proves, *N*,*N*-dimethylaniline was used for application to sample.

Analytical performance of iodide determination by GC-MS using reaction of oxidation with 2-iodosobenzoate in the presence of *N*,*N*-dimethylaniline in term of

precision and accuracy were acceptable with less than 5% relative standard deviation and 95% confidence for the analysis of certified iodide standard solution, respectively

Iodide in instant noodle seasoning was determined by the above GC-MS method using standard addition method. Performance of iodide determination in 15 seasoning samples by GC-MS was proved by paired t statistical testing with comparison method of ICP-MS and colorimetry based on Sveikina method. There are not significantly different at 95% confidence for GC-MS and ICP-MS method.

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OUTPUT

International Presentation:

W. Tiyapongpattana, W. Malethong, D. Nacapricha and P. Wilairat, *"Determination of Iodide in Seasoning Powder for Instant Noodle Using Gas Chromatography-Mass Spectrometry"* The 16th International Conference on Flow Injection Analysis Including Related Techniques, 25 -30 April 2010, Pattaya, Thailand.

International Publication:

W. Tiyapongpattana, W. Malethong, D. Nacapricha and P. Wilairat, "*Quantitative Gas Chromatography-Mass Spectrometry for Iodide in Seasoning Powder of Instant Noodle"* will be submitted to Journal of Separation Science, 2011 (Preparing Manuscripts)

APPENDIX

International Presentation:

16th INTERNATIONAL CONFERENCE ON FLOW INJECTION ANALYSIS
Including related techniques

P-013

Determination of iodide in seasoning powder for instant noodle using gas chromatography-mass spectrometry

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Abstract

A method of gas chromatography-mass spectrometry has been developed for determination of iodide (I^-) based on the oxidization of iodide to iodine (I_2) by 2-iodosobenzoate. The generated iodine then reacts with 2,6-dimethylaniline in a phosphate buffer, following by extraction of the product into organic layer for further detection by the gas chromatograph. Under the optimized condition, a satisfactory validation of data was achieved for linearity, accuracy and precision. A calibration curve (10 – 250 μgI /kg) was obtained from a plot between the area ratio of the derivative to the internal standard and the iodide concentration. The regression coefficient was higher than 0.995. The limit of detection was found to be 3 μg I/kg. Recoveries were ranged from 97% to 101%. This method can be effectively applied to determine iodide fortified in the seasoning powder of instant noodle. The fortified level is 50 μg iodide per sachet.

Keywords: Iodide; GC-MS, Derivatization, Seasoning powder

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