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A Double Extraction Method for Determination of Diphenylamine in Fruits by GC/MS

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Abstract

A double extraction method comprising ultrasound assisted QuEChERS treatment combined with hollow-fiber liquid phase micro extraction was developed for the determination of diphenylamine in various fruit samples using gas chromatography coupled to a single-quadrupole mass spectrometer. Briefly, a known portion of fruit sample underwent an ultrasound assisted QuEChERS treatment. The organic phase resulting from QuEChERS treatment of a fruit sample was diluted 5-fold with pure water followed by a hollow fiber liquid-phase micro extraction procedure for further cleaning and preconcentration. In the late procedure, the analyte was extracted from the diluted sample into a thin layer of (dodecane+10% tri-n-octylphosphine oxide) sustained in the pores of a porous hollow fiber. The analyte was back-extracted into acetonitrile located inside the lumen of the hollow fiber. Selected ion monitoring mass spectrometry was used for quantitative determination. The calibration curve was linear over the concentration range of 20-200 μg kg^{-1}. The relative recovery was in the range of 76-108% while, precision varied between 6.4-13.7%. Finally, the applicability of the joint method was tested through analysis of diphenylamine in various fruit samples.

Keywords: Diphenylamine; Ultrasound assisted QuEChERS; Hollow fiber liquid-phase micro extraction; Gas chromatography-Mass spectrometry; Fruit samples

Introduction

Diphenylamine (DPA) is an antioxidant of choice for a diverse variety of applications including propellant stabilization and preservation of apple fruit quality. DPA is also described as a naturally occurring compound in onions [1], in leaves of black and green tea, citrus fruits [2] and other plants [3,4]. It is employed for controlling browning disorder, known as scald, which may render the fruits of some apple cultivars unmarketable [1,3,5,6]. Since DPA is generally applied following harvest and prior to cold storage, qualitative and quantitative determination of DPA is of interest. An efficient sample preparation depends on the matrix, as well as on the properties and concentrations of analyte [7]. In recent years, ultrasound-assisted extraction has attracted interest, as it is an effective and rapid extraction method with extraction efficiencies comparable to that of classical techniques [8-10].

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was first developed for determination of multi-class pesticide residues in fruits and vegetables [11]. It is particularly suitable for determination of polar, middle polar and non-polar pesticides in food matrices as it is simple, inexpensive and amenable to high throughput analysis [12]. The ruggedness of QuEChERS has been evaluated with a variety of food samples [13-18]. Meanwhile, hollow fiber liquid phase micro extraction (HF-LPME) [19] involves the use of an organic solvent, typically dodecane+10% tri-n-octylphosphine oxide (TOPO) which is immobilized in the pores of a hollow fiber providing a membrane-supported liquid combined with an organic solvent, mostly acetonitril (ACN), in the lumen of the hollow fiber. HF-LPME has been reported to be an efficient and satisfactory clean-up and preconcentration procedure with excellent results [20].

The present study outlines a double extraction method in which extraction of DPA is initially carried out by ultrasound assisted QuEChERS followed by HF-LPME. Our aim was focused at studying the feasibility of HF-LPME procedure for clean-up - as an alternative to the current strategies already used along with ultrasound assisted QuEChERS treatment. To the best of our knowledge, so far this has been the first report on the extraction and measurement of DPA in fruit peel samples based on the combination of ultrasound assisted QuEChERS treatment and HF-LPME procedure.

Experimental

Chemicals and reagents

Analytical grade n-dodecane and TOPO were supplied by Fluka (Buchs, Switzerland). DPA (>99% purity), ACN, methanol, sodium chloride and anhydrous magnesium sulphate were also supplied by Merck (Darmstadt, Germany). Double distilled water was used throughout.

DPA-free apple samples were brought to the laboratory right after harvesting from a farm in the north of Tehran province (Tehran, Iran) and placed in a refrigerator upon arrival. The apples were blended with a mixer and stored at -20°C to be used as blank samples. A stock solution of 100 mg L^{-1} DPA in methanol was prepared in methanol and stored at -20°C. For constructing the matrix-matched calibration curve, ten standard solutions (in the range of 20-200 μg kg^{-1}; Calibrators (CS)) were prepared by spiking diluted proper amounts of the stock solution into the blank samples. The validation tests were carried out using three quality controls (QC) (0.5, 1.0 and 2.0 MRL) (www.wapa-association.org) -equivalent to 25, 50, 100 μg kg^{-1}. Both CS and QC samples were stored at -20°C until final analysis. At the time of analysis, the samples were left to thaw at room temperature for approximately 30 min and then underwent the whole extraction procedure.

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The Accurel Q3/2 polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness, and 0.2 μm pore size) were supplied by Membrana (Wuppertal, Germany).

GC/MS system and conditions

The gas chromatographic system consisted of an Agilent (Centerville Road, Wilmington, USA) series 7890A GC coupled to an Agilent MSD 5975C quadrupole mass spectrometer. The GC was fitted with HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) from Agilent J&W Scientific (Folsom, CA, USA). Helium (99.999%) was used as the carrier gas at the flow rate of 1.0 $mLmin^{-1}$. The following temperature program was employed for the separation: 60°C for 1 min, increased to 250°C at 5°C min^{-1} and held for 1 min, and finally increased to 300°C at 50°C min^{-1} and held for 3 min. The MS quadrupole and the MS source temperatures were set at 150 and 230°C, respectively. Data acquisition was performed in the full scan (m/z in the range of 50–700) to confirm the retention time of the analyte and in selected ion monitoring (SIM) mode (m/z=169) for quantitative determination of DPA. A dwell time of 100 ms was used with high resolution and the filament delay time was set at 3 min.

Methodology

Extraction procedure

The sample preparation procedure is characterized by two main steps: (a) solvent extraction and (b) preconcentration and clean-up. The first step is based on a sono assisted Liquid-Liquid Extraction (LLE) followed by a salting-out process, known as ultrasound assisted QuEChERS treatment. The second stage consists of a HF-LPME procedure. Our aim was to develop a methodology which was as simple as possible to measure DPA in fruit samples and one that could be easily analyzed by GC/MS. The optimum extraction procedure was as follows:

1. A 5.0 g fruit sample, which was free from the studied analyte (known as blank sample) was blended with a mixer and placed in an eppendorf vial (50 mL) and spiked with a known amount of the target analyte (from a standard solution).
2. To the same vial 15 mL of ACN was added and the mixture sonicated for 2 min using an ultrasound system (Discontinues Mode, 500 W)
3. A mixture consisting of 4 g of anhydride magnesium sulfate and 1 g of sodium chloride was added to the above solution. This mixture was shaken vigorously for 2 min then underwent centrifugation at 3000 rpm for 5 min.
4. The organic upper layer (2 mL) was decantated and transferred into an 11 mL glass vial previously filled with 8 mL of pure water (i.e., 5-fold dilution)
5. A 25 μL HPLC syringe and a conventional medical syringe needle were inserted through the silicon septum, the former served to introduce the acceptor solution (ACN) into the hollow fiber prior to extraction and collect this solution after extraction, while the latter needle was utilized for supporting the hollow fiber. Then, an 8.8-cm length of hollow fiber was placed between the two needle ends and subsequently immersed in an extracting organic solvent (dodecane+10% TOPO) for several seconds to ensure that the pores of the hollow fiber membrane were filled with the extracting solvent. The diffused solvent in the lumen of the fiber was removed by blowing air using a 5 mL syringe, and 25 μL of the organic acceptor solution was carefully inserted into the lumen of the fiber using the micro syringe. Following that, the fiber was placed in the aqueous sample solution stirred at 750 rpm.
6. The analyte was extracted from the diluted sample into a thin layer of organic solvent (dodecane+10% TOPO) sustained in the pores of a porous hollow fiber. Then, it was back-extracted into 25 μL volume of the organic acceptor solution (ACN) located inside the lumen of the hollow fiber.
7. Once the extraction time (30 min) was reached, the acceptor solution was flushed into a micro tube. Of which, 1.0 μL was injected directly into the GC/MS [19].

Enrichment factor

In order to examine the Enrichment factor (EF) of each analyte, a series of standard solutions in ACN were prepared and directly injected into the GC-MS. Then, peak area was plotted against analyte concentration after the whole extraction process. The EF was then calculated as the slope ratio of the extraction process calibration to that of the non-extraction (directly injected) curve.

Method validation

The method was validated via the following parameters: Selectivity, linearity range, lower limit of quantification (LLOQ), precision, relative recovery as well as the matrix effect.

Selectivity of the method was demonstrated by injecting the diluted stock solution of analyte. In addition, 16 blank samples were analyzed to check whether there were any interfering peaks in the chromatogram that could complicate the analysis.

Relative recovery (RR) was determined using the following equation:

$$\% RR=100 \times (C_{found}-C_{real})/C_{added}$$

Where, C_{found} and C_{added} are the concentrations of DPA in the real/blank sample after and before the addition of the working solutions.

Linearity of the method was determined by constructing the matrix-matched calibration curve at ten concentration levels over the range of 20-200 μg kg^{-1} for DPA. Calibration curves were constructed by plotting the analyte signal obtained, which was the average of three measurements, against the respective concentration of the analyte. The LLOQ was measured based on the following definition: The lowest concentration at which the relative error falls between -20% and +20% and the precision with a coefficient of variation (RSD %) of ± 20% obtained under five measurements.

And finally, the matrix effect (ME) of the assay was evaluated by comparing the average of the peak areas of the DPA (n=3) at three different concentration levels (0.5, 1.0 and 2.0 MRL).

Prepared in pure water (A) and the blank samples (B), respectively. ME was then calculated by using the following formula:

$$ME (\%)=100 \times B/A$$

Results and Discussion

Optimization of ultrasound assisted QuEChERS treatment

To find the optimum conditions for DPA extraction in the fruit samples, initially sonication time was tested in the range of 0-3 min. The result revealed that the recovery of DPA was slightly enhanced, when the ultrasonic treatment was increased from 0 to 2 min. However, the recovery remained almost unchanged beyond 2 min (date not shown). Therefore, 2 min was taken as the optimum sonication time. The volume of ACN was adjusted at 15 mL, so that the extracted upper layer after the ultrasound assisted QuEChERS treatment was 2 mL. Other

factors such as the amount of sample (5.0 g), the sonication parameters (50 KHz, 110 W), the type of extraction solvent (ACN) and the salt content ($MgSO_4$: NaCl; 4:1 g) were maintained invariable due to being reported in various papers [13-18,21]. It is noted that the DPA recovery after ultrasound assisted QuEChERS treatment from the spiked apple samples at 50 μg kg^{-1} level was taken as the response of the optimization experiments.

All in all, the optimal conditions of the ultrasound assisted QuEChERS treatment are as follows: Sonication time, 2 min; Extraction solvent volume, 15 mL ; Extraction solvent, ACN ; and salts amounts ($MgSO_4$: NaCl ; 4:1 g).

Optimization of HF-LPME treatment

The modified HF-LPME apparatus was introduced first in detail by Ghambarian et al. [19], which was implemented in this study with little modification. The optimal conditions for HF-LPME used were as follows: Stirring rate, 750 rpm; extraction time, 30 min.

Analytical performance

According to the results obtained, there were no interfering peaks, originating from the fruit sample matrix or the chemicals and reagents used, at the same retention time of the analyte in any of the 16 blank samples studied in the selectivity experiments (data not shown). In practice, more compounds were co-extracted from the matrix of fruit in ultrasound assisted QuEChERS treatment alone than those extracted by the joint QuEChERS/HF-LPME method. This explains the reason for high selectivity of the joint method, which is attributed to the application of the highly selective clean-up, low cost (no need for expensive QuEChERS sorbent for clean-up) method (i.e., HF-LPME).

The results also demonstrated a fair linearity for DPA over the concentration range of 20-200 μg kg^{-1} with a correlation coefficient of 0.9885. LLOQ was determined by monitoring the lower concentration meeting the criteria discussed in the method validation section, which is clearly more appropriate than the theoretically calculated value. Additionally, the average EF value of DPA was determined to be 45 (n=3).

Matrix components can inhibit or enhance analyte signal. Since matrix components may affect the analyte signal and may also be dependent on the nature of the analyte, the use of an isotope internal standard is the best way to compensate for any matrix affects [22]. Unfortunately, for DPA, an isotopic internal standard was not available; as an alternative, matrix-matched calibration was used [23-28]. The matrix effect results (ME%) were determined to be 104 ± 10, 108 ± 9 and 111 ± 13 (n=3) for 0.5, 1.0 and 2.0 MRL, respectively. Based on the obtained results, little positive matrix effect was observed, indicating that the matrix had almost negligible impact on the whole extraction efficiency due to the implementation of HF-LPME as an excellent clean-up procedure.

Analysis of real samples

The applicability of the joint method for the measurement of DPA in some commercial fruit samples was investigated. The samples, which included apple, pear and peach, were purchased from local stores. From the six commercial samples analyzed, none were found to have been treated or so-called contaminated with DPA (i.e., no response was obtained at the retention time of DPA). The proposed method was successfully applied to the analysis of DPA in the above-mentioned fruit samples spiked at three different QC levels as discussed in section 3.1. The relative recovery (RR) tests were performed at each QC level, and the recovery value considered was the average of three measurements. As can be seen in Table 1, the relative recoveries varied between 76 and 108%.

The RSD% results based on five similar measurements were within the range of 6.4-13.7%, as presented in Table 1. On the whole, the tabulated data shows relatively good recoveries along with a high precision indicating that the applied double extraction method is highly efficient for the measurement of DPA in various fruit samples. Figure 1 depicts the GC/MS chromatogram of DPA following the ultrasound assisted QuEChERS/HF-LPME treatment at LLOQ level (20 μg kg^{-1}) in the commercial apple peel sample.

Comparison of this study with other related studies

The results obtained from this study were compared with other related studies regarding the analysis of DPA in several fruit samples. As indicated in Table 2, the application of a simple clean-up and further preconcentration step using HF-LPME yielded comparable data to other related studies (Table 2).

Conclusion

Ultrasound assisted QuEChERS treatment combined with modified hollow-fiber liquid phase micro extraction method was developed for the measurement of diphenylamine (DPA) in various fruit samples using GC/MS analysis. The linearity range, lower limit of quantification (LLOQ), precision, relative recovery and matrix effect

Sample	Added[a]	Found[a]	RSD (%)	RR (%)
	25	21	12.2	84
Apple 1	50	54	9.5	108
	100	83	8.5	83
	25	22	11.4	88
Apple 2	50	46	7.3	92
	100	89	6.4	89
	25	26	9.4	104
Apple 3	50	41	10.1	82
	100	106	8.4	106
	25	20	7.8	80
Pear 1	50	42	8.9	84
	100	82	7.0	82
	25	19	8.3	76
Pear 2	50	47	9.4	94
	100	85	6.8	85
	25	19	13.7	76
Peach	50	39	11.5	78
	100	80	10.0	80

a: Concentration (μg kg^{-1})

Table 1: Analysis of diphenylamine in various fruit samples.

Reference	RR (%)	LR[a]	RSD (%)	Analytical method
[24]	96-105	0.3-5.1 μg ml^{-1}	<6.4	TLC scanner
[25]	78-104	0.25-5 mg kg^{-1}	<3	Fluorometric optosensor
[26]	77-114	-	<14	LC and GC/MS
[27]	98-102	10-100 μg kg^{-1}	2-6	Spectrofluorimetry
[6]	[b]113	-	6.3	LC/UV and MS
[28]	[b]88.5	-	4.9	GC/MS
This work	76-108	20-200 μg kg^{-1}	6.4-13.7	GC/MS

a: Range of linearity; b: Average recovery

Table 2: Comparison of the applied method with other related studies.

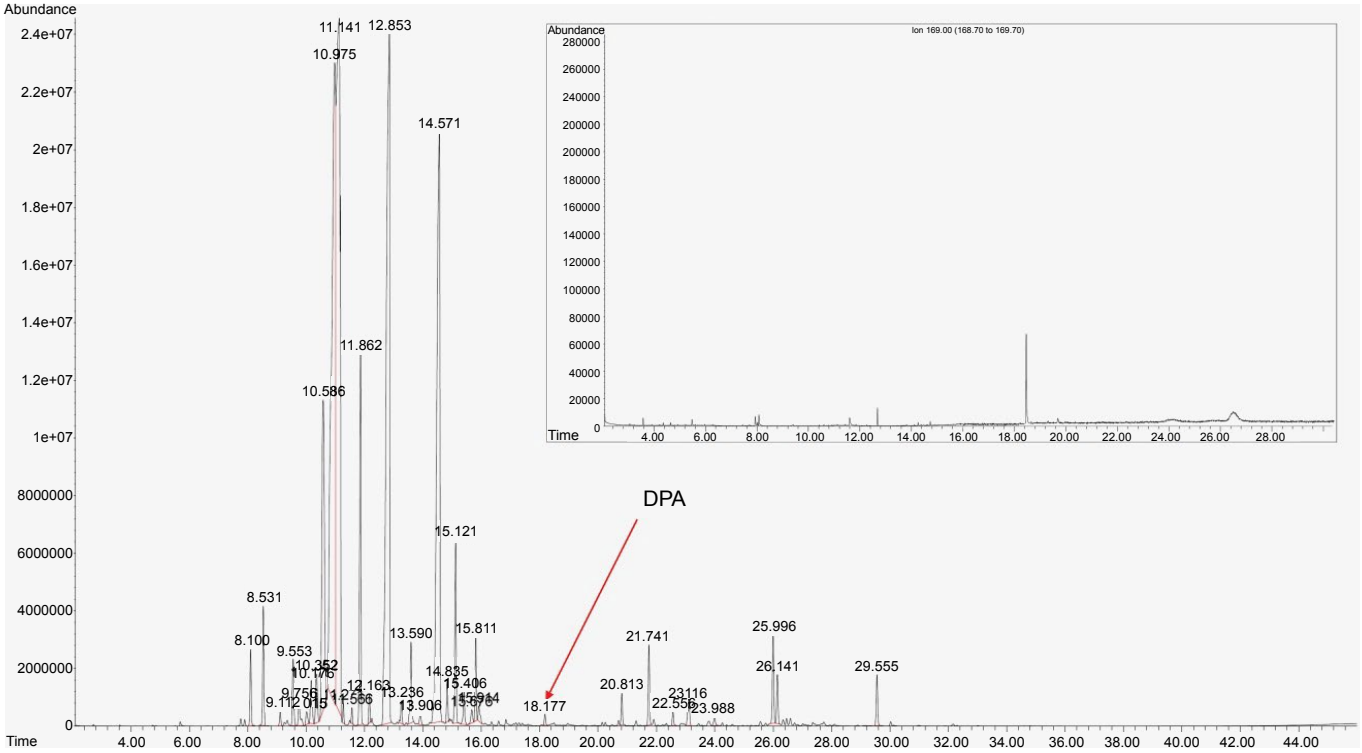

Figure 1: GC/MS chromatogram of DPA following the ultrasound assisted QuEChERS/HF-LPME treatment at LLOQ level (20 µg kg^{-1}) in the commercial apple sample.

were all fully evaluated. The method is simple, rapid, efficient and easy to conduct. The results obtained from the analysis of real spiked samples showed a good analytical performance in all studied fruit matrices, which highlights the use of HF-LPME as an effective clean-up treatment once jointly applied with conventional methods. Finally, this modified method could be easily implemented in routine analysis.

Disclosure Statement

No potential conflict of interest was reported by the authors.

References

1. Karawya MS, Abdel Wahab SM, El-Olemy MM, Farrag NM (1984) Diphenylamine, an antihyperglycemic agent from onion and tea. J Nat Prod. 47: 775-780.
2. Drzyzga O (2003) Diphenylamine and derivatives in the environment: A review. Chemosphere. 53: 809-818.
3. Nose M, Nakatani Y, Yamanishi T (1971) Studies on the flavor of green tea. Part IX. Identification and composition of intermediate and high boiling constituents in green tea flavor. Agri biol chem. 35: 261-271.
4. Wang LQ, Qin GW, Chen SN, Li CJ (2001) Three diterpene glucosides and a diphenylamine derivative from Pieris formosa. Fitoterapia 72: 779-787.
5. Saad B, Haniff NH, Saleh MI, Hashim NH, Abu A, Alic N (2004) Determination of ortho-phenylphenol, diphenyl and diphenylamine in apples and oranges using HPLC with fluorescence detection. Food Chem. 84: 313-317.
6. Rudell DR, Mattheis JP, Fellman JK (2005) Evaluation of diphenylamine derivatives in apple peel using gradient reversed-phase liquid chromatography with ultraviolet visible absorption and atmospheric pressure chemical ionization mass selective detection. J Chromatogr A. 108: 202-209.
7. Rodrigues SA, Caldas SS, Primel EG (2010) A simple, efficient and environmentally friendly method for the extraction of pesticides from onion by matrix solid-phase dispersion with liquid chromatography–tandem mass spectrometric detection. Anal Chim Acta 678: 82-89.
8. Picó Y (2013) Ultrasound-assisted extraction for food and environmental samples. Tr Anal Chem. 43: 84-99.
9. Awad TS, Moharram HA, Shaltout OE, Asker D, Youssef MM (2012) Applications of ultrasound in analysis, processing and quality control of food: A review. Food Res Int. 48: 410-427.
10. Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: A review. Tr Food Sci Technol. 21: 323-331.
11. Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ (2003) Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. J AOAC Int. 86: 412-431.
12. Koesukwiwat U, Sanguankaew K, Leepipatpiboon N (2008) Rapid determination of phenoxy acid residues in rice by modified QuEChERS extraction and liquid chromatography–tandem mass spectrometry. Anal Chim Acta; 626: 10-20.
13. Lehotay SJ, Son KA, Hyeyoung KH, Koesukwiwat U, Fu W, et al. (2010) Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. J Chromatogr A. 1217: 2548-2560.
14. Wilkowska A, Biziuk M (2011) Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chem. 125: 803-812.
15. Cieślik E, Sadowska RA, Ruiz JMM, Surma ZM (2011) Evaluation of QuEChERS method for the determination of organochlorine pesticide residues in selected groups of fruits. Food Chem. 125: 773-778.
16. Hou, X, Han M, Dai X, Yang X, Yi S (2013) A multi-residue method for the determination of 124 pesticides in rice by modified QuEChERS extraction and gas chromatography–tandem mass spectrometry. Food Chem. 138: 1198-1205.
17. Marchis D, Ferro GL, Brizio P, Stefania SS, Abete MC (2012) Detection of pesticides in crops: A modified QuEChERS approach. Food Contr; 25: 270-273.
18. Nguyen TD, Yu JE, Lee DM, Lee GA (2008) multiresidue method for the determination of 107 pesticides in cabbage and radish using QuEChERS sample preparation method and gas chromatography mass spectrometry. Food Chem. 110: 207-213.
19. Ghambarian M, Yamini Y, Esrafili A, Yazdanfar N, Moradi MA (2010) new concept of hollow fiber liquid–liquid–liquid microextraction compatible with gas

chromatography based on two immiscible organic solvents. J Chromatogr A. 1217: 5652-5658.

20. Arvand M, Bozorgzadeh E, Shariati S (2013) Two-phase hollow fiber liquid phase microextraction for preconcentration of pyrethroid pesticides residues in some fruits and vegetable juices prior to gas chromatography/mass spectrometry. J Food Comp Anal. 31: 275-283.

21. Park J, Choi J, Abd El-Aty AM, Kim BM, Oh J, et al. (2011) Simultaneous multiresidue analysis of 41 pesticide residues in cooked foodstuff using QuEChERS: Comparison with classical method. Food Chem. 128: 241-253.

22. Anastassiades M, Maštovska K, Lehotay SJ (2003) Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides. J Chromatogr A. 1015: 163-184.

23. Kirchner M, Huškova R, Matisova E, Mocak J (2008) Fast gas chromatography for pesticide residues analysis using analyte protectants. J Chromatogr A. 1186: 271-280.

24. Baghel A, Joshi S, Amlathe S (2012) Disposable sensors for the determination of diphenylamine with ninhydrin as a choromogenic reagent. J Chem Pharma Sci. 4: 2704-2711.

25. García RJF, Ortega BP, Molina DA (2005) Rapid determination of diphenylamine residues in apples and pears with a single multicommuted fluorometric optosensor. J Agri Food Chem. 53: 9874-9878.

26. Nguyen TD, Yun MY, Lee GHA (2009) multiresidue method for the determination of 118 pesticides in vegetable juice by gas chromatography mass spectrometry and liquid chromatographyetandem mass spectrometry. J Agri Food Chem. 57: 10095-10101.

27. Farokhcheh A, Alizadeh N (2013) Determination of diphenylamine residue in fruit samples using spectrofluorimetry and multivariate analysis. LWT Food Sci Technol. 54: 6-12.

28. Xiao-Gang C, Xiao-Zhong H, Hui-Yuan Y (2005) Determination of 266 pesticide residues in apple juice by matrix solid-phase dispersion and gas chromatography–mass selective detection. J Chromatogr A. 1063: 201-210.