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Development of a multiresidue method for determination of 86 multiclass pesticides in pineapple juice using gas chromatography tandem mass spectrometry

S. DAS, A. KUNDU, A. BHATTACHARYYA, D. SINGHA, S. SAHA, A. BHATTACHARYYA AND S. ROY

Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya Mohanpur-741252, Nadia, West Bengal

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ABSTRACT

A simple, rapid and modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was developed for single stroke analysis of 86 multiclass pesticides in pineapple juice using Gas chromatography tandem mass spectrometry (GC-MS/MS). From the preliminary trial of three methods (viz. Method I, II and III) Method I, due to better mean recovery percentage of individual pesticides, was selected and validated based upon selectivity, sensitivity, linearity, precision, accuracy, Limit of Detection (LOD) and Limit of Quantification (LOQ) criteria. The correlation coefficient (r2) of all pesticides ranged between 0.97 - 0.99 and the average recovery values within 70 - 120 % with intra laboratory repeatability (RSDr) below 20 % and appreciable Horwitz ratio in between 0.5 to 2 at LOQ level; proved that this method is sufficiently accurate, repeatable and therefore can be effectively utilized for accurate profiling of pesticide residues in export samples.

Keywords **:** GC-MS/MS, method validation, multiresidue analysis, pesticides, pineapple juice

Pineapple, *Ananas comosus* (L.) is the third most important subtropical fruit crop having extraordinary nutritional profile, which is primarily due to ample supply of vitamin C, carbohydrates, crude fibre, minerals *viz*. calcium, potassium and antioxidants, such as ascorbic acid, flavonoids and other phenolic compounds (Brat *et al*., 2004; Mhatre *et al*., 2009). Again, the crude extract of pineapple contains a proteolytic enzyme bromelain, which helps in inhibition of bronchitis (Neubauer, 1961); osteoarthritis (Mojcik and Shevach, 1997); cardiovascular diseases by inhibiting blood platelet aggregation. Nevertheless, such an extraordinary nutritional quality of this fruit is rapidly augmenting it's worldwide demand either in the form of raw dessert or processed foodstuff *i.e*. juice. Besides, extremely shorter shelf life of raw fruits after harvest is indirectly emphasizing it's export in the form of juice having an extended shelf life period of nearly 6 months.

But, the pineapple production in India is threatened by several pest infestations, and with an increased level of product contamination through extensive use of a wide group of pesticides to protect the crop against infestations, indirect contamination by introduction of pesticides from non-point sources and Persistant Organic Pollutants (POPs) having excessive resistance to environmental degradation; a thorough pre-export screening of these samples towards a very lower level of pesticide residues is extremely necessary. Besides, according to the CODEX general standard for fruit juices and nectars (CODEX STAN 247-2005: Section 6.1), the products should also comply with a prescribed Maximum Residue Limit (MRL) for acceptance in international market. In this regards, the objective of our present experiment is to develop a validated multiclass multiresidue method, which is analyst friendly *i.e*. fast, economic, easy to perform and involves sufficiently broad spectrum of analytes with appreciable selectivity, precision and recovery values.

MATERIALS AND METHODS

Pineapple juice samples were collected from local supermarkets and were tested further to confirm to be free from contamination with any of the selected pesticides prior to selection as a matrix for the experiment. We selected 86 GC amenable pesticides, including 48 insecticides, 19 herbicides, 15 fungicides and 4 acaricides; of which majority pesticides are directly applied in pineapple orchards.The Certified Reference Materials (CRM) of test pesticides were purchased from Sigma-Aldrich, having purity values > 98%. Organic solvents of HPLC grade *viz.* ethyl acetate and acetonitrile were supplied by J. T. Baker. A GC-MS/MS system (GC system: Agilent Technologies 7890A; MS system: Agilent Technologies 7000) equipped with HP-5 MS column (Agilent J&W) and Triple quadrupole mass spectrometer (Software: Masshunter Workstation Software B.05.00) was included for instrumental analysis.

Individual primary stock solutions of 86 pesticides were prepared from the Certified Reference Materials

Email: roysankhabckv@gmail.com

(CRMs) using ethyl acetate solvent, from which two working mixtures of 1 µg ml^{-1} and 5 µg ml^{-1} were prepared. The 5 µg ml^{-1} mixture was utilized to spike the samples, while the 1 µg ml^{-1} mixture was used for preparation of matrix matched calibrations. A six point calibration curve *viz*. 5, 10, 20, 50, 100 and 250 ng ml^{-1} was prepared in ethyl acetate solvent. The recovery experiments were carried out by fortifying the samples (10 g) @ 10, 50 and 100 ng ml^{-1} in three replicates.

Regarding optimization of GC condition, the initial temperature was held at 70°C with a hold time of 2.5 minutes, followed by five ramps were used for effective separation of the selected analytes. This programme resulted in a total time of 38.857 minutes in a single chromatographic run. During entire analysis the temperature of auxiliary heater was maintained at 280ºC. The mass spectrophotometric parameters included an electron impact (EI) ionisation of eluted analytes at -70 eV energy with the source temperature of 230ºC, MS-I and MS-II quad temperatures at 150ºC.

Ten gram of juice sample was taken in 50ml polypropylene centrifuge tubes and extracted following three different methods. In the first method (*i.e*. Method I) the samples were added with 10ml ethyl acetate solvent and subjected to vortex for 1 minute. Then a combined salt system of 1.5g sodium chloride and 5g activated sodium sulphate were added; immediately vortexed thoroughly for 2 minutes; followed by rotospin for 15 minutes. The samples were then centrifuged at 5000 rpm for 5 minutes and 5ml aliquot from the upper layer was collected in 10 ml graduated tubes. The modifications in solvent and salt systems, used for Method II were 10 ml of acetonitrile and a combination of 1.5g sodium chloride and 5g activated magnesium sulphate respectively. However, a buffered system was included in Method III, in which 10 ml of acetonitrile along with 100 ìl acetic acid were utilized as an extracting solvent and a combination of 1.5 g sodium acetate and 5 g activated magnesium sulphate were used as salt system. The remaining procedures were kept unchanged in both the cases. Regarding clean up, 1.5 ml of the collected supernatant from Method I was transferred into 2 ml microcentrifuge tubes containing 50 mg PSA and 150 mg muffled anhydrous sodium sulphate; votexed for 1 minute and centrifuged in minicentrifugal system at 5000 rpm for 10 minutes. The supernatant was filtered with syringe filter, containing 13 mm nylon 6, 6 membrane prior to instrumental analysis. However for Method II and III, before clean up the extracted analytes were transferred from acetonitrile to ethyl acetate by evaporation in nitrogen evaporator, followed by reconstitution with ethyl acetate solvent accordingly to keep the concentration of individual analytes unchanged.

Finally, the analytical method was validated according to the single laboratory validation approach of SANTE guidelines (SANTE/11945/2015). The selectivity of the method was evaluated by injecting extracted blank samples. The sensitivity of the method was confirmed in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ) for which a signal to noise ratio of 3:1 and 10:1 respectively were accepted. Linearity of calibration included six levels ranging from 5 ng ml^{-1} to 250 ng ml^{-1}. Precision was expressed in terms of intra laboratory repeatability (RSD_r) and Horwitz ratio at LOQ level. Accuracy was measured by analysing the sample of known concentration and comparing the measured value to the 'true' value. A signal to noise ratio of 3:1 and 10:1 were accepted to determine LOD and LOQ respectively.

RESULTS AND DISCUSSION

A time efficient chromatographic method was prepared by appropriate optimization of both the gas chromatographic and mass spectrometric parameters. The temperature programming was constituted of stepwise elevation of temperature starting from 70°C to 290°C with involvement of 5 ramps and 5 well classified time segments. The elution pattern of the analytes can be classified in 3 simple categories: early eluting compounds (methamidophos and dichlorvos), mid eluting compounds (mainly organochlorines and organophosphorus) and later eluting compounds (mainly pyrethroids). Elution of methamidophos and dichlorvos within first 8 minute runtime in segment 1 indicated a very less interaction of these two compounds with column stationary phase. An increment of temperature from 180°C to 200°C in ramp 2 with a lower rate of 5°C $minute^{-1}$ and a corresponding hold time of 3 minutes resulted in elution of 22 analytes and effectively separated very closely eluting compounds which was prominent in case of â and ã HCH isomers. In ramp 3 and ramp 4 temperatures were elevated to 20°C at a rate of 5 and 7°C $minute^{-1}$ respectively, which eluted nearly 34 compounds (major OP and OC compounds) especially the DDT and DDE isomers. The final ramp in which the temperature was increased 50°C at a rate of 10°C $minute^{-1}$ eluted nearly 28 compounds including pyrethroids. A longer hold time of 12 minutes at 290°C was introduced at last to ensure the absence of any retention in the column.The mass spectrophotometric parameters included an electron impact (EI) ionisation of eluted analytes at -70 eV energy, 230ºC source temperatureand the MS-I and MS-II quad temperatures at 150ºC. The flow of quench gas, i.e. helium and the collision gas, *i.e*. nitrogen were maintained at 2.25 and 1.5 ml $minute^{-1}$ respectively. The MS/MS parameters of individual compound are presented below.

Table 1: MS/MS parameters for individual target analytes

Compound name	Rt (min)	Quantifier transition	CE	Qualifier transition	CE
3-keto carbofuran	8.67	177.70 > 163.00	10	136.70 > 43.20	15
Acetochlor	11.01	174.70 > 132.10	10	222.90 > 147.20	5
Alachlor	14.22	188.10 > 160.20	10	160.00 > 132.10	10
á-cypermethrin	27.66	163.00 > 91.00	10	163.00 > 127.00	5
á-endosulfan	18.57	240.50 > 206.00	5	194.90 > 160.00	5
á-HCH	11.47	216.90 > 181.00	5	218.90 > 183.00	5
Anilophos	24.46	225.60 > 157.00	15	225.60 > 183.90	5
Atrazine	11.88	214.90 > 58.10	10	214.90 > 200.20	5
Benalaxyl	22.14	148.00 > 77.00	20	148.00 > 105.10	35
Benthiocarb	15.39	100.00 > 72.00	5	124.90 > 89.00	15
â-endosulfan	20.75	194.90 > 158.90	15	206.90> 172.00	10
â-HCH	12.03	216.90 > 181.10	15	181.00 > 145.00	5
Bifenox	24.39	340.50 > 188.80	10	189.10 > 126.00	20
Bifenthrin	24.04	180.80 > 165.10	10	181.20 > 166.20	25
Bitertanol	26.20	170.10 > 141.10	20	170.10 > 115.00	40
Buprofezin	19.99	104.70 > 77.10	20	105.80 > 77.00	20
Butachlor	18.77	175.70 > 147.20	15	159.70 > 132.10	15
Carbaryl	9.48	144.00 > 115.10	20	144.00 > 116.10	10
Carboxin	19.83	142.70 > 43.20	15	234.60 > 143.10	10
Carfentrazone ethyl	22.21	339.90 > 311.90	10	339.90 > 309.90	10
Chlorfenvinphos	17.48	266.50 > 159.00	15	268.50 > 161.00	15
Chlorpyriphos	15.82	196.90 > 169.00	15	198.90 > 171.00	15
Chlorpyriphos methyl	13.97	124.90 > 47.00	15	124.90 > 78.90	5
Clodinafop propergyl	22.50	265.60 > 91.30	20	237.60 > 129.90	25
Cyfluthrin	27.15	162.90 > 90.90	15	162.90 > 127.00	5
Cyhalofop butyl	25.14	256.20 > 120.10	10	120.10 > 91.00	15
ä-HCH	12.78	217.00 > 181.10	5	181.10 > 145.10	15
Deltamethrin	29.96	181.00 > 152.10	15	252.90 > 93.00	25
Diazenon	12.51	137.10 > 84.00	10	137.10 > 54.00	20
Dichlorvos	7.43	184.90 >93.00	5	109.00 > 79.00	10
Dicofol	15.90	139.00 > 111.10	10	249.60 > 139.00	10
Dieldrin	19.59	262.50 > 193.00	40	78.80 > 51.10	20
Dimethoate	11.69	86.80 > 46.10	15	142.90 >111.00	10
Edifenphos	22.16	172.90 > 109.00	5	108.90 > 65.10	15
Endosulfan sulphate	22.25	273.80 > 238.90	15	271.90 > 237.00	15
Ethion	21.36	152.90> 96.90	10	124.90 > 96.90	10
Etofenprox	27.81	163.00 > 135.10	10	163.00 > 107.10	20
Etrimphos	12.93	181.00 > 153.10	5	168.00 > 153.10	5
Fenamedone	24.29	238.00 > 237.20	10	268.00 > 180.20	20
Fenarimol	25.53	219.00 > 107.10	10	251.00 > 139.10	10
Fenazaquin	24.35	145.00 > 117.10	10	160.00 > 145.20	5
Fenitrothion	14.95	125.10 > 47.00	15	125.10 > 79.00	5
Fenthion	15.73	278.00 > 109.00	15	124.90 > 47.00	10
Fenvalerate	28.72	167.00 > 125.10	5	208.90 > 141.10	15
Fluchloralin	12.58	325.80 > 62.90	15	306.00 > 263.90	10
Flusilazole	20.03	233.00 > 165.10	15	233.00 > 91.00	20
ã-HCH	12.20	216.90 > 181.00	5	181.00 > 145.00	15
Haloxyfop methyl	18.32	316.00 > 91.00	20	375.00 > 316.00	10
Hexythiazox	18.00	183.60 > 149.00	10	155.60 > 112.00	5
ë-cyhalothrin	25.43	180.90 > 152.00	5	197.00 > 141.00	10
Malathion	15.37	126.90 > 99.00	5	172.90 > 99.00	15

Contd...

Table 1 Contd.

Compound name	Rt (min)	Quantifier transition	CE	Qualifier transition	CE
MCPA methyl	9.96	140.70 > 77.10	20	213.60 > 155.10	10
Metalaxyl	14.40	205.70 > 132.10	5	234.00 > 146.10	20
Methamidophos	7.26	140.70 > 95.00	5	140.70 > 64.00	25
Methyl parathion	13.96	124.70 > 79.00	5	108.70 > 79.00	10
Methyl paraxon	12.68	108.70 > 79.10	5	229.60 > 106.20	20
Metominostrobin	19.47	190.70 > 160.10	10	195.70 > 77.00	20
Monolinuron	11.92	60.90 > 46.10	10	126.00 > 99.00	15
o,p-DDD	19.89	235.00 > 165.20	20	237.00 > 165.20	20
o.p-DDE	18.32	246.00 > 176.20	30	248.00 > 176.20	30
o.p-DDT	21.23	235.00 > 165.20	20	237.00 > 165.20	20
Oxyfluorfen	20.00	252.00 > 146.00	20	252.00 > 196.00	30
p,p-DDD	21.12	234.90 > 165.10	20	236.90 > 165.20	20
p,p-DDE	19.57	246.10 > 176.20	30	315.80 > 246.00	15
p,p-DDT	22.39	235.00 > 165.20	20	237.00 > 165.20	20
Paclobutrazole	18.34	124.70 > 89.10	10	235.70 > 125.10	20
Parathion	15.84	96.70 > 47.10	5	290.90 > 109.00	10
Penconazole	17.15	158.60 > 89.10	15	248.00 > 192.10	25
Pendimethalin	17.12	251.80 > 162.20	10	251.80 > 161.10	15
Permethrin	26.48	182.90 > 153.10	10	183.10 > 165.10	10
Phorate	11.31	121.00 > 65.00	10	121.00 > 47.00	30
Phorate sulfone	15.59	152.70 > 97.10	10	124.70 > 97.00	5
Phorate sulfoxide	15.29	153.00 > 96.90	10	96.90 > 64.90	20
Phosalone	24.86	182.00 > 111.00	15	182.00 > 102.10	15
Pretilachlor	19.58	162.10 > 147.20	10	162.10 > 132.20	20
Propergite	23.04	134.70 > 107.20	15	134.70 > 77.00	30
Propiconazole	22.48	172.60 > 145.10	20	172.60 > 109.00	20
Propaxur	10.39	110.00 > 63.00	25	110.00 >64.00	15
Pyraclostrobin	26.10	388.10 > 194.00	10	388.10 > 163.00	25
Quinalphos	17.61	146.00 > 118.00	10	146.00 > 91.00	30
Quizalofop ethyl	27.62	371.50 > 299.10	20	298.50 > 254.80	15
Simazine	17.59	168.00 > 70.00	10	112.00 > 58.10	10
Tau fluvalinate	29.12	250.00 > 55.00	20	250.00 > 200.00	15
Tetraconazole	16.12	336.00 > 218.40	30	336.00 > 128.00	40
Tetradifon	24.62	110.90 > 75.00	25	148.70 > 93.10	20
Triadimefon	15.93	208.00 > 181.10	5	208.00 > 111.00	20

Table 2: Mean recovery (%) of 3 methods in pineapple juice fortified with pesticides @ 100 ng ml^{-1} level

Compound name	Avg. recovery % (100 ng ml^{-1})		
	Method I	Method II	Method III
3-keto carbofuran	101.59	80.17	94.43
Acetochlor	80.93	59.41	44.61
Alachlor	92.26	72.30	60.55
á-cypermethrin	78.37	106.15	153.87
á-endosulfan	98.16	74.26	53.77
á-HCH	92.35	24.44	46.20
Anilophos	93.20	68.20	82.15
Atrazine	80.40	30.47	34.69
Benalaxyl	83.67	96.67	156.28
Benthiocarb	106.87	21.85	28.64
â-endosulfan	95.12	26.88	34.26

Contd...

Table 2 Contd.

Compound name	Avg. recovery % (100 ng ml^{-1})		
	Method I	**Method II**	**Method III**
â-HCH	97.94	26.47	35.19
Bifenox	84.70	27.01	34.48
Bifenthrin	109.12	101.15	108.37
Bitartanol	88.20	71.56	70.56
Buprofezin	102.58	83.11	95.32
Butachlor	94.01	84.14	46.02
Carbaryl	80.18	70.76	48.69
Carboxin	78.32	80.74	59.69
Carfentrazone ethyl	93.24	88.79	112.24
Chlorfenvinphos	89.31	36.64	60.80
Chlorpyriphos	101.38	92.51	104.70
Chlorpyriphos methyl	104.30	85.75	59.18
Clodinafop propergyl	84.02	77.06	58.71
Cyfluthrin	83.02	71.74	70.38
Cyhalofop butyl	92.89	80.15	92.73
ä-HCH	102.16	72.28	76.21
Deltamethrin	93.39	70.54	74.21
Diazenon	91.58	69.50	69.97
Dichlorvos	107.49	55.74	58.44
Dicofol	91.56	87.02	84.44
Dieldrin	94.75	74.51	53.19
Dimethoate	106.47	79.66	56.37
Edifenphos	100.13	82.34	58.01
Endosulfan sulphate	92.99	68.07	60.64
Ethion	75.95	100.00	80.44
Etofenprox	113.09	99.46	106.07
Etrimphos	100.56	71.54	65.10
Fenamedone	92.15	95.49	83.47
Fenarimol	93.64	95.81	81.83
Fenazaquin	98.53	85.74	96.11
Fenitrothion	91.58	77.34	70.53
Fenthion	110.23	104.95	73.28
Fenvalerate	101.84	84.25	73.07
Fluchloralin	104.20	49.90	51.51
Flusilazole	92.58	89.13	88.10
ã-HCH	96.55	51.40	58.50
Haloxofop methyl	87.89	98.35	93.07
Hexythiazox	78.35	94.16	87.51
ë-cyhalothrin	104.85	57.13	56.34
Malathion	101.73	92.91	80.75
MCPA methyl	95.59	59.77	60.99
Metalaxyl	90.40	86.58	76.75
Methamidophos	103.45	109.54	93.39
Methyl parathion	101.51	68.83	62.88
Methyl paraxon	106.05	71.21	69.77
Metominostrobin	103.68	74.64	73.14
Monolinuron	103.04	89.07	76.98
o,p,-DDD	107.59	82.92	71.77
o.p-DDE	100.81	81.04	69.31
o.p-DDT	84.78	81.31	69.84

Contd...

Table 2 Contd.

Compound name	Avg. recovery % (100 ng ml^{-1})		
	Method I	**Method II**	**Method III**
Oxyflurofen	78.47	103.18	89.77
p.p-DDD	99.51	105.43	89.87
p.p-DDE	97.31	114.47	97.25
p.p-DDT	95.52	112.11	99.12
Paclobutrazole	97.84	104.81	86.43
Parathion	94.31	102.36	84.19
Penconazole	81.40	109.76	85.48
Pendimethalin	89.32	99.68	82.66
Permethrin	81.56	85.38	113.49
Phorate	78.21	116.08	94.27
Phorate sulfone	91.27	77.91	87.79
Phorate sulfoxide	96.01	93.72	77.26
Phosalone	93.78	97.23	87.52
Pretilachlor	102.77	98.03	96.09
Propargite	81.07	91.04	75.38
Propiconazole	87.75	105.31	89.56
Propaxur	101.58	101.29	81.23
Pyraclostrobin	100.15	102.05	77.79
Quinalphos	105.78	124.30	117.96
Quizalofop-p-ethyl	101.93	79.09	45.58
Simazine	96.53	92.96	89.65
Tau fluvalinate	94.48	110.70	89.31
Tetraconazole	100.21	98.25	86.48
Tetradifon	97.83	120.50	83.72
Triadimefon	100.54	104.78	84.96

Table 3: Average recovery percentage (RSD$_r$) of the test pesticides at different levels of fortifications and Horwitz Ratio (10 ppb level) in Pineapple juice

Compound name	Avg. recovery % (RSD$_r$)			r^2	Hor	LOQ
	10 ng ml^{-1}	**50 ng ml^{-1}**	**100 ng ml^{-1}**		**Rat.**	**(ng ml^{-1})**
3-keto carbofuran	99.60 (17.89)	91.44 (9.73)	101.59 (9.20)	0.99	0.56	10
Acetochlor	83.90 (14.83)	78.38 (11.36)	80.93 (8.63)	0.99	0.46	10
Alachlor	70.80 (9.70)	94.86 (15.32)	92.26 (16.04)	0.98	0.30	10
á-cypermethrin	99.60 (14.53)	80.88 (9.30)	78.37 (7.43)	0.99	0.45	10
á-endosulfan	90.50 (14.07)	82.78 (5.23)	98.16 (7.50)	0.99	0.44	10
á-HCH	74.60 (11.73)	80.68 (15.38)	92.35 (11.45)	0.99	0.37	10
Anilophos	86.00 (20.27)	95.90 (10.70)	93.20 (15.01)	0.97	0.63	10
Atrazine	103.90 (16.56)	95.78 (15.57)	80.40 (9.09)	0.99	0.52	10
Benalaxyl	102.50 (18.10)	82.46 (17.17)	83.67 (14.31)	0.99	0.57	10
Benthiocarb	88.00 (12.87)	92.82 (3.58)	106.87 (3.61)	0.99	0.40	10
â-endosulfan	98.40 (16.86)	85.22 (8.36)	95.12 (11.43)	0.99	0.53	10
â-HCH	86.40 (14.84)	75.96 (5.28)	97.94 (5.41)	0.99	0.46	10
Bifenox	76.01 (10.45)	86.38 (11.03)	84.70 (13.96)	0.98	0.37	10
Bifenthrin	94.30 (16.57)	92.94 (6.79)	109.12 (4.13)	0.98	0.52	10
Bitartanol	—	89.44 (11.68)	88.20 (8.56)	0.98	0.36	50
Buprofezin	105.90 (13.48)	89.48 (5.06)	102.58 (13.40)	0.98	0.42	10
Butachlor	100.60 (17.24)	78.26 (2.97)	94.01 (16.62)	0.99	0.54	10
Carbaryl	90.30 (13.81)	78.16 (4.56)	80.18 (6.95)	0.97	0.43	10
Carboxin	92.00 (14.54)	80.70 (7.76)	78.32 (8.44)	0.99	0.45	10

Contd...

Table 3 Contd.

Compound name	Avg. recovery % (RSD_r)			r^2	Hor	LOQ
	10 ng ml^{-1}	50 ng ml^{-1}	100 ng ml^{-1}		Rat.	(ng ml^{-1})
Carfentrazone ethyl	—	95.86 (7.85)	93.24 (9.85)	0.98	0.39	50
Chlorfenvinphos	—	88.22 (9.81)	89.31 (4.23)	0.98	0.43	50
Chlorpyriphos	94.80 (14.36)	88.52 (4.67)	101.38 (12.00)	0.99	0.45	10
Chlorpyriphos methyl	111.90 (13.05)	91.82 (18.81)	104.30 (11.04)	0.99	0.41	10
Clodinafop propergyl	96.40 (14.32)	79.68 (11.96)	84.02 (6.41)	0.99	0.45	10
Cyfluthrin	96.20 (15.03)	96.44 (6.47)	83.02 (12.01)	0.99	0.47	10
Cyhalofop butyl	93.00 (13.09)	83.28 (14.73)	92.89 (14.80)	0.99	0.41	10
ä-HCH	92.50 (13.45)	75.32 (5.85)	102.16 (3.17)	0.99	0.42	10
Deltamethrin	86.90 (13.40)	96.52 (13.90)	93.39 (20.17)	0.99	0.42	10
Diazenon	85.50 (9.89)	95.94 (8.84)	91.58 (14.47)	0.99	0.31	10
Dichlorvos	84.20 (13.62)	86.92 (8.65)	107.49 (2.72)	0.99	0.43	10
Dicofol	86.90 (10.17)	79.96 (9.03)	91.56 (25.08)	0.98	0.32	10
Dieldrin	96.90 (14.94)	87.58 (13.43)	94.75 (23.17)	0.98	0.47	10
Dimethoate	101.10 (14.89)	74.44 (17.61)	106.47 (3.03)	0.99	0.47	10
Edifenphos	86.00 (12.72)	95.28 (7.79)	100.13 (11.50)	0.99	0.40	10
Endosulfan sulphate	105.80 (15.23)	94.10 (8.58)	92.99 (6.34)	0.99	0.48	10
Ethion	92.00 (13.79)	95.02 (9.67)	75.95 (6.32)	0.97	0.43	10
Etofenprox	94.50 (15.12)	82.68 (9.99)	113.09 (8.37)	0.98	0.47	10
Etrimphos	89.00 (13.78)	79.46 (13.69)	100.56 (10.42)	0.99	0.43	10
Fenamedone	89.90 (11.90)	89.92 (5.97)	92.15 (7.25)	0.99	0.37	10
Fenarimol	90.00 (12.12)	93.80 (4.30)	93.64 (6.51)	0.97	0.38	10
Fenazaquin	81.30 (14.49)	105.22(16.87)	98.53 (15.16)	0.99	0.45	10
Fenitrothion	83.70 (10.01)	89.72 (5.54)	91.58 (9.46)	0.99	0.31	10
Fenthion	86.30 (11.26)	88.78 (15.94)	110.23 (3.21)	0.99	0.35	10
Fenvalerate	90.00 (14.47)	87.92 (12.58)	101.84 (6.80)	0.99	0.45	10
Fluchloralin	96.50 (15.33)	87.20 (14.80)	104.20 (3.85)	0.99	0.48	10
Flusilazole	86.33 (9.62)	99.06 (6.33)	92.58 (16.13)	0.98	0.34	10
ã-HCH	91.00 (11.30)	76.14 (14.75)	96.55 (13.50)	0.99	0.35	10
Haloxifop methyl	80.93 (15.58)	83.04 (17.50)	87.89 (17.02)	0.97	0.52	10
Hexythiazox	86.00 (12.40)	93.48 (12.65)	78.35 (18.83)	0.97	0.39	10
ë-cyhalothrin	94.70 (9.55)	83.04 (5.76)	104.85 (2.71)	0.99	0.30	10
Malathion	84.10 (14.58)	80.98 (18.77)	101.73 (2.98)	0.99	0.46	10
MCPA methyl	78.40 (15.74)	78.14 (14.25)	95.59 (12.05)	0.98	0.49	10
Metalaxyl	100.90 (15.76)	86.26 (16.51)	90.40 (14.25)	0.99	0.49	10
Methamidophos	—	94.58 (14.46)	103.45 (4.37)	0.99	0.47	50
Methyl parathion	87.10 (14.50)	82.02 (11.83)	101.51 (15.89)	0.99	0.45	10
Methyl paraxon	82.55 (11.56)	86.30 (14.38)	106.05 (15.43)	0.99	0.48	10
Metominostrobin	110.00 (10.42)	90.74 (10.31)	103.68 (13.06)	0.98	0.33	10
Monolinuron	94.60 (9.58)	78.60 (11.27)	103.04 (14.52)	0.99	0.30	10
o,p,-DDD	83.90 (11.64)	97.74 (9.26)	107.59 (6.31)	0.99	0.36	10
o.p-DDE	79.20 (14.46)	82.82 (12.64)	100.81 (8.21)	0.99	0.45	10
o.p-DDT	88.50 (16.13)	80.80 (12.21)	84.78 (10.49)	0.97	0.50	10
Oxyflurofen	94.50 (17.71)	93.84 (3.77)	78.47 (2.14)	0.99	0.55	10
p.p-DDD	88.30 (14.80)	88.70 (9.86)	99.51 (14.75)	0.99	0.46	10
p.p-DDE	80.60 (12.47)	85.44 (14.67)	97.31 (14.25)	0.99	0.39	10
p.p-DDT	83.50 (12.40)	89.94 (14.03)	95.52 (9.10)	0.99	0.39	10
Paclobutrazole	91.89 (10.36)	86.40 (18.73)	97.84 (14.64)	0.98	0.31	10
Parathion	93.10 (10.61)	95.36 (8.93)	94.31 (18.72)	0.99	0.33	10
Penconazole	85.40 (11.93)	107.90 (7.56)	81.40 (8.21)	0.98	0.37	10
Pendimethalin	73.60 (10.87)	90.20 (13.10)	89.32 (6.02)	0.98	0.34	10

Contd...

Table 3 Contd.

Compound name	Avg. recovery % (RSD$_r$)			r^2	Hor	LOQ
	10 ng ml^{-1}	50 ng ml^{-1}	100 ng ml^{-1}		Rat.	(ng ml^{-1})
Permethrin	106.80 (15.02)	89.12 (17.08)	81.56 (9.45)	0.97	0.47	10
Phorate	95.70 (11.06)	99.28 (11.11)	78.21 (9.86)	0.99	0.35	10
Phorate sulfone	91.70 (10.79)	95.90 (13.26)	91.27 (4.82)	0.99	0.34	10
Phorate sulfoxide	85.80 (9.74)	87.28 (11.00)	96.01 (12.75)	0.99	0.30	10
Phosalone	103.50 (12.64)	90.08 (10.49)	93.78 (18.00)	0.98	0.40	10
Pretilachlor	90.30 (9.91)	94.44 (11.93)	102.77 (11.82)	0.99	0.31	10
Propargite	107.30 (14.55)	93.18 (7.07)	81.07 (9.92)	0.99	0.45	10
Propiconazole	86.80 (15.68)	78.94 (17.32)	87.75 (15.18)	0.99	0.49	10
Propaxur	72.40 (10.48)	83.90 (10.70)	101.58 (14.61)	0.99	0.33	10
Pyraclostrobin	—	94.26 (11.93)	100.15 (12.75)	0.99	0.35	50
Quinalphos	81.80 (17.61)	96.02 (15.04)	105.78 (8.00)	0.97	0.55	10
Quizalofop ethyl	82.40 (16.91)	95.34 (9.87)	101.93 (13.17)	0.99	0.53	10
Simazine	93.20 (16.90)	89.52 (18.39)	96.53 (14.39)	0.99	0.53	10
Tau fluvalinate	100.01 (12.14)	84.76 (4.91)	94.48 (12.37)	0.98	0.38	10
Tetraconazole	114.20 (12.20)	78.44 (14.19)	100.21 (6.39)	0.99	0.38	10
Tetradifon	103.40 (11.56)	85.66 (12.95)	97.83 (7.57)	0.99	0.36	10
Triadimefon	84.90 (17.88)	96.66 (17.35)	100.54 (14.50)	0.99	0.56	10

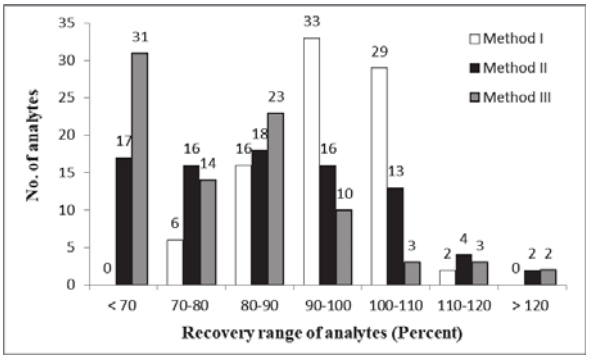

Fig. 1: Recovery distribution of 86 analytes in three methods at 100 ng ml^{-1}spike level

Compairing the recovery results of individual analytes at 100 ng ml^{-1} level, it was observed that the average recovery percentage of all the analytes were ranged within 70 to 120 per cent in Method I (Table 2). However, in Method II, 19 compounds showed either enhancement or suppression in their recovery values; which increases upto 33 in Method III (Fig. 1). Nevertheless, Method I which involves ethyl acetate as the extraction solvent, showed better performance; which is again in accordance with similar results obtained for low fat, sugar rich substrates, extracted with ethyl acetate (Mastovaska and Lehotay, 2004; Savant *et al*., 2010) and therefore Method I was selected for further exploitation through validation.

Comparing the chromatograms of blank matrix and spiked matrix, the absence of a signal above a signal to noise ratio of 3 at the retention times of the target compounds confirmed that the method is free of interferences. Besides, the majority of the population showed LOQ value of 10 ng ml^{-1} except 5 pesticides *viz*. Bitertanol, Carfentrazone ethyl, Chlorfenvinphos, Methamidophos and Pyraclostrobin; proving that the method is sensitive. The correlation coefficient (r^2) values were in the range of 0.97 – 0.99 (Table 3) for individual analytes, proving that the method is sufficiently linear. Besides, the calculated intra laboratory repeatability values (RSD$_r$) for every single compounds at three spiked levels were below 20 % (Table 3), confirming that the method is precise enough. The Horwitz ratio of all the 86 pesticides calculated at LOQ level was within the acceptable range of 0.5 to 2.0 (Table 3) as proposed by AOAC guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals (2012), which ensured that the method has satisfactory repeatability and is sufficiently rugged.Again, the recovery percentage at LOQ level of all 86 pesticides was in the range of 70-120 %,thereby proving the method is accurate. 81 out of 86 pesticides showed a satisfactory signal to noise ratio above 10:1 at 10 mg ml^{-1} (Table 3), confirming this concentration level as the limit of quantification of these compounds. However, only 5 pesticides *viz*. Bitertanol, Carfentrazone ethyl, Chlorfenvinphos, Methamidophos and Pyraclostrobin showed a higher LOQ level of 50 ng ml^{-1} (Table 3) below which a satisfactory signal to noise ratio could not be achieved for these compounds.

This experiment reveals an improvised multiresidue method for single stroke analysis of 86 pesticides in pineapple juice. A brief single chromatographic run time

of 38.857 minutes provided optimum separation for all the target analytes under consideration along with appreciable recovery of individual pesticides in between 70 - 120 %, RSD_r values below 20 % and appreciable linearity within 0.97-0.99; proved that the method can be effectively utilized for routine analysis as well as a guideline to know the pesticide residue status before shipment for export.

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