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Short communication

Simultaneous analysis of neuroendocrine tumor markers by HPLC-electrochemical detection

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ABSTRACT

A validated, high pressure liquid chromatographic (HPLC) method for simultaneous quantitation of urinary catecholic acids 4-hydroxy-3-methoxymandelic acid (HMMA) (vanylmandelic acid) (VMA), 4-hydroxy-3-methoxyphenylacetic acid (HVA) and 5-hydroxyindole-3-acetic acid (5-HIAA) was developed. Sample preparation involved liquid–liquid extraction of acidified urine, containing iso-HMMA (IS) as internal standard, with ether, evaporation of the organic extract, followed by reconstitution of the residue in phosphate buffer at pH 3.3. After reversed-phase HPLC at 35 °C and separation on a Lichrospher 125 mm × 4 mm C_{18} column (5 μm particle size) with phosphate buffer (pH 3.5)–acetone (950:50, v/v) as eluent, quantitation is achieved by electrochemical detection using coulometric detection at a potential of +350 mV. The method was successfully applied to routine diagnosis of neuroblastoma, carcinoid syndrome and pheochromocytoma.

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1. Introduction

The metabolism of catecholamines norepinephrine (NE), epinephrine (E), and dopamine (DA) leads to the major metabolites vanylmandelic acid (VMA), 4-hydroxy-3-methoxymandelic acid (HMMA) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) (Fig. 1).

Neuroblastoma, a neoplastic disease of early childhood, is the third most common cancer in children. Measurements of these metabolites serve as a screening test for neuroblastoma [2,3].

The metabolism of serotonin to 5-hydroxyindole-3-acetic acid (5-HIAA) (Fig. 1) is a diagnostic marker in urine for the "carcinoid syndrome" [4].

Although pheochromocytoma [1] is characterized by high catecholamine production, there is also generally increased urinary excretion of the corresponding metabolites, HMMA and HVA.

Our previous HPLC-fluorescence assay [5], for these metabolites showed inconsistent retention times, low sensitivity, especially for HVA, and frequent bias (inaccuracy) judged by results from the external quality assurance program, RCPA-AACB, to which our laboratory subscribes.

Several methods [5–8] for the measurement of catecholic acids have been developed. Some disadvantages of such methods include interferences, long times, prior derivatisation, cost implications or the requirement for technically skilled personnel.

Both HVA and VMA contain the electroactive vanillyl moiety. Liquid chromatography with electrochemical detection is thus the method of choice for their determination.

Although ECD is not the method of choice for 5-HIAA, we attempted to incorporate the assay of the latter into the same assay for the neuroblastoma markers which would be useful for simultaneous screening for carcinoid syndrome, without the need for an additional assay procedure for the latter.

This paper presents a novel, validated HPLC assay method for simultaneously measuring urinary HMMA, HVA, and 5-HIAA, using electrochemical detection, which is amenable to routine analysis in the clinical setting for subsequent diagnosis of these neoplastic diseases.

Ethics approval for the study (reference number: BE 148/09) was waivered by the Biomedical Research Ethics Committee (BREC), University of KwaZulu-Natal (UKZN).

2. Experimental

2.1. Chemicals

(DL)-4-Hydroxy-3-methoxy-mandelic acid (HMMA), (DL)-3-hydroxy-4-methoxy-mandelic acid (iso-HMMA) (internal standard) (IS), 4-hydroxy-3-methoxy-phenylacetic acid (HVA), 5-

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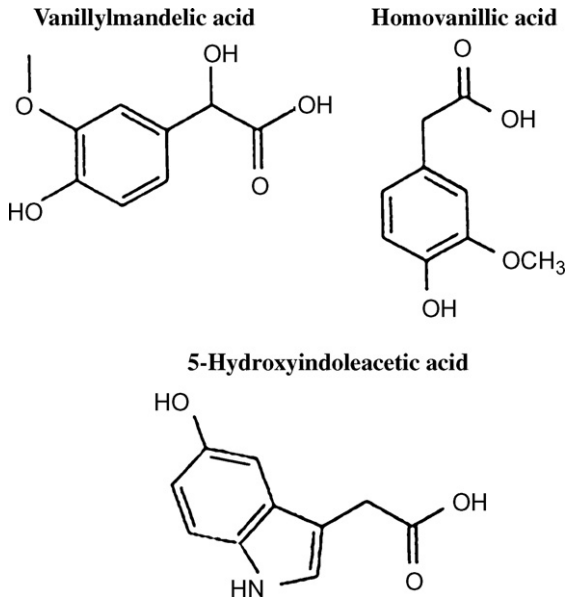

Fig. 1. Structure of the acids.

hydroxyindole-acetic acid (5-HIAA), sodium phosphate monobasic, EDTA di-sodium salt di-hydrate and acetone, were obtained from Sigma (St. Louis, USA). Diethyl ether and methanol were obtained from Burdick & Jackson. Ammonium sulphate, propan-2-ol, concentrated hydrochloric acid and ortho-phosphoric acid were obtained from Merck. Nitrogen gas (11 kg, 99.999%) was obtained from Air Liquid (South Africa). BIO-RAD Lyphochek Quantitative Urine Control (Level 1 and Level 2) catecholic acid quality control material was obtained from Bio-Rad Laboratories (Hercules, CA). External quality control material was obtained from the RCPAA EQA Program, Australia, to which our laboratory subscribes annually. All solvents and chemicals were of HPLC grade.

2.2. Instrumentation and conditions

Water was obtained from a Milli-RO PLUS 30 water purification system (Millipore, USA). HPLC analysis was performed on a PerkinElmer (PE) Series 200 HPLC system (USA), equipped with a PE Series 200 binary pump, PE Series 200 Peltier column oven, PE Series 200 Peltier Controller sample tray, PE Series 200 Autosampler (loop size 200 μl), PE Series 200 Vacuum degasser, PE Series 600 LINK Chromatography Interface, PE NCI 900 Network Chromatography Interface and programmable ESA Coulochem II Multi-Electrode electrochemical detector (equipped with Coulometric cells: Model 5011 Analytical Cell and Model 5021 Conditioning Cell) (Chelmsford, USA) with the α-hydrogen/palladium as the reference electrode. A TurboChrom Workstation (Version 6.2) software was used for data collection, processing and monitoring. Chromatographic separation was accomplished with a LichroCART LicroSpher 100 RP-18e (125 mm × 4 mm I.D., 5 μm size) cartridge and corresponding LichroCART LiChrospher 100 RP-18e (4 mm × 4 mm, 5 μm) utilized as a guard column (Merck).

2.3. Mobile phase

The mobile phase consisted of 50.00 mM sodium phosphate monobasic/0.08 mM EDTA (in distilled water (18.2 MΩ) adjusted to pH 3.5 with phosphoric acid:water (1:1, v/v)–acetone (950:50, v/v), filtered 3–5 times through a 0.22 μm filter membrane. The mobile phase flow rate, isocratic, through the column, maintained at 35 °C, was 0.7 ml/min, with a run time of 20 min.

2.4. Phosphate buffer (pH 3.3)

50.00 mM sodium phosphate monobasic/0.08 mM EDTA (in distilled water (18.2 MΩ) was adjusted to pH 3.3 with phosphoric acid:water (1:1, v/v) and filtered once through a 0.22 μm filter membrane.

2.5. Standard solutions

External Standard preparation—stock standard: separate stock standards of HMMA, IS (iso-HMMA), 5-HIAA and HVA were prepared in methanol at a concentration of 0.500 mg/ml.

Working external stock was prepared daily from the stock standards: 100 μl HMMA, 200 μl IS, 75 μl 5-HIAA, 100 μl HVA, evaporation to dryness with nitrogen gas and reconstitution in phosphate buffer (5.000 ml).

Working external standard was prepared daily in phosphate buffer from working external stock (80 μl) and 4.920 ml phosphate buffer.

Working internal standard was prepared daily by evaporating stock internal standard solution (0.500 mg/ml) (200 μl) to dryness with nitrogen gas and immediate reconstitution in phosphate buffer (3.125 ml).

2.6. Urine samples

For adults, 24-h urine samples from subjects, were collected in amber bottles containing 100 ml 2 M HCl. For children, a random urine was collected and adjusted to pH 1–3 immediately with concentrated hydrochloric acid and the creatinine concentration was determined. Samples were stored at 4 °C if necessary for 1–2 days or at −20 °C for longer periods.

2.7. Sample preparation

400 μl urine sample (or Biorad Level 1 control, or external quality assurance sample), 100 μl working IS solution (32 μg/ml) (3.2 μg) and 150 μl concentrated HCl was added to 0.55 g ammonium sulphate in a KIMAX tube. For the Biorad Level 2 control, 300 μl of phosphate buffer was first added to the salt in the KIMAX tube, followed by 100 μl of control sample. The capped tube was manually extracted (90 s) with 5 ml ether and centrifuged at 4000 rpm for 2 min at room temperature. The ether phase was transferred with Pasteur pipette to a glass vial. The extraction and centrifugation step was repeated once. The combined ether extracts (10 ml) were evaporated to dryness with nitrogen gas at room temperature. Immediately phosphate buffer pH 3.3 (10.000 ml) was added to the residue followed by vortex-mix. Approximately 1 ml of sample was then filtered through a micron (0.45 μm) filter-syringe into an amber HPLC vial (1.8 ml). Ten microlitres of sample extract (or External Standard) was injected into the HPLC system for analysis.

3. Results and discussion

3.1. Development and optimization of analytical aspects of method

Common HPLC methods for catecholic acids make use of fluorimetric detection systems [5,6]. Comparatively fewer HPLC-electrochemical detection-based assays are reported [9,10].

During initial development, all patient and control samples were initially run in parallel using both the previous fluorescence and the electrochemical detection-based assay. Sample volume, concentrated HCl volume, mass of ammonium sulphate and ether volume were maintained. The volumes of stock solutions of analytes used

for standard preparations were optimized with respect to observed response factors and sensitivity on the resulting chromatograms.

The initial extraction method involved liquid–liquid extraction of acidified urine, containing ammonium sulphate and IS, with ether, followed by back-extraction into phosphate buffer pH 7, based on Gironi's [5] fluorescent method. A sample extract volume of 10 μl for injection into the HPLC equipment was initially, at column temperature of 35 °C, used and was not subsequently varied. Their reported C_{18} Nova Pak column and eluent was initially used. Chromatography was rather unsatisfactory regarding resolution, peak shape and sensitivity.

Whilst maintaining the initial liquid–liquid extraction procedure with ether, and use of the Licrospher C_{18} column (5 μm particle size), which was found to work well for our urine catecholamine assay, with column temperature of 35 °C, the following parameters were varied: potential of the analytical cell, mobile phase composition (monochloroacetic acid, sodium octane sulphonate, citric acid, diethylamine, acetonitrile, sodium phosphate, acetone) and pH, flow rate, use of dilute acids (acetic acid, HCl) and their pH, for standard preparation and reconstitution, use of nitrogen gas and compressed air for the evaporation of organic extracts, to determine optimum analytical parameters like resolution, peak height, retention time, sensitivity, recovery and accuracy. The latter two was initially optimized by use of the known target values of the commercial Biorad Control material. The peak response for IS was appropriately reduced by variation of the volume of phosphate buffer (pH 3.3) added to the dried organic acid extracts. A 5 ml volume was initially used but it was eventually found that a volume of 10 ml was optimum to prevent overload of HPLC column and detector. Hydrodynamic voltammetry was then used to optimize the analytical potential with respect to the signal response (sensitivity) obtained for HMMA.

It was found that use of sodium phosphate adjusted to pH 3.5 with 5% acetone as eluent, at flow rate of 0.7 ml/min, with column temperature 35 °C, and an analytical potential of +350 mV, was optimum for all target acids.

The usual validation parameters were then determined as outlined above.

3.2. Method validation criteria

3.2.1. Analytical potential

The analytical potential was optimized by hydrodynamic voltammetry in Scan mode using single electrode operation for the peak height response of HMMA using a standard mixture of the three acids and internal standard in phosphate buffer. The following conditions gave optimum signal:noise response: guard cell: +350 mV, DC Channel 1—*E*: 0 mV, *R*: 100 nA, filter: 5 s, Ch1 output: 1 V, offset: 5%; DC Channel 2—*E*: +350 mV, *R*: 100 nA, filter: 5 s, Ch2 output: 1 V, offset: 5%. A potential of +350 mV was found to be optimum for analysis.

3.2.2. Choice of peak area versus peak height

Peak height integration was found to be superior to peak area integration. The CV% of 10 within-day consecutive injections were 0.24%, 0.21%, 0.70% and 0.44% for HMMA, IS, 5-HIAA and HVA, respectively, using an External Standard solution.

3.2.3. Specificity

The resulting chromatograms were essentially free from endogenous interference. The retention times averaged 2.788 ± 0.082 min, 3.359 ± 0.115 min, 11.587 ± 0.455 min and 16.319 ± 0.689 min for HMMA, IS, 5-HIAA and HVA, respectively. Fig. 2 shows a standard solution of acids. Peak shape and resolution between HMMA and IS were satisfactory.

3.2.4. Sensitivity

Normal pooled urine was spiked with all three target analytes at concentrations of 4.94, 9.88, 19.75 and 39.50 μmol/L. A plot of spiked analyte concentration versus ratio of analyte/IS response afforded the following regression equations: $r^2 = 0.961124$ (averaged over 3 days), $y = 0.055922x + 0.202809$, $y = 0.056542x - 0.17067$, $y = 0.050715x - 0.13802$ for HMMA, 5-HIAA and HVA, respectively.

3.2.5. Linear range

Standard solutions prepared in phosphate buffer were used. The data were fitted to a line by the equation $y = ax + b$ where y is the peak height, b, the intercept and a the slope. Regression analysis showed good linearity. The correlation coefficients, determined over 5 days, averaged 0.99184 (SD = 0.001221, CV% = 0.12) for all three acids for a concentration range of 0.04–39.50 μmol/L ($y = 18.1x + 0.8$), 0.08–39.50 μmol/L ($y = 11.3x - 1.0$) and 0.31–39.50 μmol/L ($y = 8.8x + 5.0$) for HMMA, 5-HIAA and HVA, respectively.

3.2.6. Limit of detection (LOD) and limit of quantitation (LOQ)

Using standards in phosphate buffer, at a minimum signal:noise ratio of 3.0:1, the limit of detection was found to be 0.15 μmol/L (30 μg/L), 0.03 μmol/L (59 μg/L) and 0.08 μmol/L (15 μg/L) for HMMA, 5-HIAA and HVA, respectively. The LOQ was evaluated as the minimum concentration detectable at a signal:noise ratio of ≥10:1 to be approximately 0.62 μmol/L (123 μg/L), 1.23 μmol/L (235 μg/L) and 0.62 μmol/L (113 μg/L) for HMMA, 5-HIAA and HVA, respectively. Both parameters were determined from the average values obtained over 5 days.

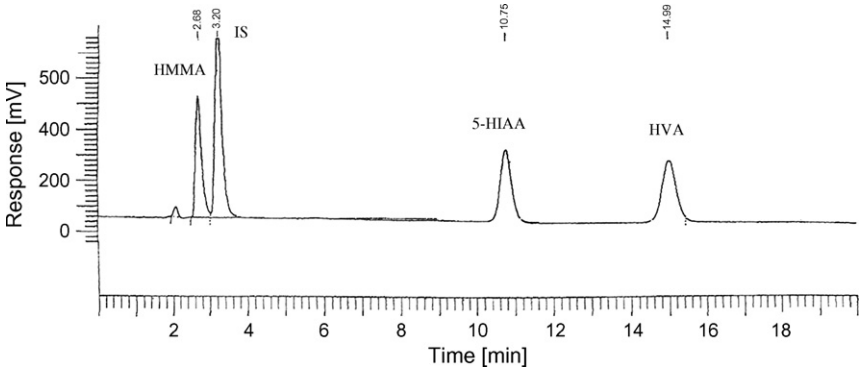

Fig. 2. Chromatogram of an External Standard: HMMA (RT 3.2 min), IS (RT 3.2 min), 5-HIAA (RT 10.8 min), and HVA (RT 15.0 min).

Table 1
Accuracy and precision using Biorad control material (normal and abnormal).

Acid	Mean found (μmol/L)	Accuracy (%)	Bias (%)	CV (%)	Biorad target value/range[a] (μmol/L)
Reproducibility					
Level 1 (Lot 62181)					
HMMA	16.7	111.33	+11.33	0.60	11.0–20.0 (15.0)
5-HIAA	18.4	90.20	−9.80	4.89	15.7–25.1 (20.4)
HVA	13.2	114.78	+14.78	1.52	7.7–15.4 (11.5)
Mean		105.44		2.34	
Level 2 (Lot 62182)					
HMMA	75	108.70	+8.70	2.63	52–86 (69)
5- HIAA	141	95.58	−4.42	2.98	116–177 (146)
HVA	100	120.48	+20.48	2.00	63–100 (83)
Mean		108.25		2.54	
Repeatability					
Level 1 (Lot 62191)					
HMMA	16.6	110.68	+10.68	5.42	11.0–20.0 (15.0)
5-HIAA	16.8	76.36	−23.64	9.52	16.7–27.2 (22.0)
HVA	10.7	97.27	−2.73	14.02	7.1–14.8 (11.0)
Mean		94.77		9.65	
Level 2 (Lot 62192)					
HMMA	82	118.84	+18.84	4.88	52–86 (69)
5-HIAA	141	90.39	−9.61	13.48	123–189 (156)
HVA	100	114.94	+14.94	6.00	70–105 (87)
Mean		108.06		8.12	

[a] 2 SD range (mean value in parenthesis).

3.2.7. Stability

All stock standards were stable at 4 °C for 30 days.

3.2.8. Carry-over evaluation

A blank phosphate buffer sample was injected after running of extracted, spiked urine samples and of standards of the acids in phosphate buffer. In all cases, the observed signal responses were virtually non-detectable or negligible.

3.2.9. Accuracy

The accuracy was determined by analyzing internal quality control material, external quality assurance material and by determining recovery of added acids, as standards in phosphate buffer, to pooled normal urine over the entire linear range.

For Biorad-urine controls, due to the target values exceeding the detector linear range for the Level 2 quality control material, a 1/4 dilution in phosphate buffer of control material was used for assay. Individual mean accuracy was 112.34%, 88.13% and 118.87% for HMMA, 5-HIAA and HVA, respectively (Table 1). Overall accuracy averaged 106.45% for all three acids.

For the external quality assurance samples (Australia), 25 five EQA samples were analyzed together with batches of routine patient samples. We receive a total of 12 lots of EQA samples, for a 12-month period, each lot containing 3 samples. One specified lot, as per the program instructions, is assayed together with a routine sample batch on a monthly basis. The data were fitted to a line by the equation $y = ax + b$ where y is the peak height, b, the intercept and a the slope. Regression analysis showed good linearity. The correlation statistics observed were as follows—HMMA: $r^2 = 0.998257$, $a = 1.09129$, $b = -1.37208$; 5-HIAA: $r^2 = 0.986749$, $a = 1.036953$, $b = -5.63698$; HVA: $r^2 = 0.997423$, $a = 1.200449$, $b = -1.18985$. The correlation coefficients averaged 0.994143 (SD = 0.006416955, CV% = 6.45) with a mean accuracy of 102.51% and mean bias of 2.77% for all three acids. The corresponding t-test values were 0.4244, 0.1884, and 0.9580 for HMMA, 5-HIAA and HVA, respectively. All t-values were less than 2.0106 at a P value of 0.050 showing that observed assay values were not significantly different from the EQA median values.

The observed mean recoveries (accuracy) at four different levels, repeated over 3 days, covering the entire linear range was 101 ± 10% (CV = 3.21%), 80 ± 11% (CV = 13.66%) and 107 ± 3% (CV = 5.05%) for HMMA, 5-HIAA and HVA, respectively and was on average equal to 96 ± 8% (CV = 7%) for all three acids (Table 2). The corresponding recovery for the IS was 101.4%, 98.7%, 97.3% and 100.6% at 4.94 μmol/L, 9.88 μmol/L, 19.75 μmol/L and 39.50 μmol/L, respectively and overall 99.50% (±1.84).

3.2.10. Precision

Precision was determined for both inter- and intra-day variability by analysis of internal quality control samples and pooled patient samples at normal and pathological concentrations. Repeatability was studied by replicate analysis for $n = 10$ aliquots. Reproducibility was determined by assaying samples on $n = 10$ different days. Due to the target values exceeding the detector linear range for the Level 2 quality control material, a 1/4 dilution in phosphate buffer of control material was used for assay. Pathological samples were diluted in phosphate buffer appropriately. Results are summarized for internal quality control in Table 1.

1. *Use of Biorad urine control material*: Due to the target values exceeding the detector linear range for the Level 2 quality control material, a 1/4 dilution in phosphate buffer of control material was used for assay. The within-day (inter-day) precision was 0.60%, 4.89% and 1.52% for Level 1 and 2.63%, 2.98%, 2.00% for Level 2 for HMMA, 5-HIAA and HVA, respectively. The corresponding day-to-day (intra-day) precision was 5.42%, 9.52% and 14.02% for Level 1 and 4.88%, 13.48%, 6.00% for Level 2 for HMMA, 5-HIAA and HVA, respectively. The repeatability precision averaged 3.61% and the reproducibility precision averaged 8.89% for both levels.
2. *Real samples*: For pooled, normal samples, the assay values were 20, 14–15 and 23–24 μmol/L with CV% of 0%, 7%, 4% for within-day precision and 3%, 12% and 6% for day-to-day precision for HMMA, 5-HIAA and HVA, respectively. For pooled, pathological samples, the assay values were 77–79, 74, 87–89 μmol/L with CV% of 1%, 3%, 2% for within-day precision and 1%, 8% and 7% for day-to-day precision for HMMA, 5-HIAA and HVA, respec-

Table 2
Recovery and accuracy of catecholic acids.

Acid	Concentration added (μmol/L)	*n*	Concentration found Mean ± SD (μmol/L)	Recovery (Accuracy) Mean ± SD (%)	CV (%)
HMMA	39.50	10	44.32 ± 0.45	112.21 ± 1.14	1.02
	19.75	10	20.20 ± 0.30	102.25 ± 1.51	1.48
	9.88	10	11.07 ± 3.30	102.26 ± 3.05	2.98
	4.94	10	4.37 ± 0.32	88.48 ± 7.37	7.37
Overall mean				101.30	
SD				3.21	
5-HIAA	39.50	10	37.45 ± 3.70	94.81 ± 9.35	9.86
	19.75	10	14.96 ± 2.26	75.76 ± 11.42	15.07
	9.88	10	7.70 ± 1.45	77.97 ± 14.69	18.84
	4.94	10	3.47 ± 0.38	70.35 ± 7.64	10.86
Overall Mean				79.72	
SD				13.66	
HVA	39.50	10	41.00 ± 0.41	103.80 ± 1.03	0.99
	19.75	10	20.74 ± 0.40	105.02 ± 2.11	1.91
	9.88	10	10.67 ± 1.12	107.96 ± 11.31	10.48
	4.94	10	5.49 ± 0.37	111.25 ± 6.81	6.81
Overall mean				107.10	
SD				5.05	

tively. The within-day precision on average was 4% and 2% for the normal and pathological urine, respectively and the day-to-day precision was on average 6% and 5%, respectively for all three acids.

3.3. Application

3.3.1. Neuroblastoma

Cytology for a 2-year infant suggested a nephroblastoma. The urine catecholamines assay values were NE = 0.78, *E* = 0.330, DA = 3.8 μmol/mmol creatinine. The normal reference range is NE < 0.28, *E* < 0.045, and DA < 2.2 μmol/mmol creatinine. The corresponding urine catecholic acids assay values were, after 1 in 2 dilution of urine, HMMA = 36 and HVA = 74 μmol/day (Fig. 3). The normal reference range is HMMA < 20 and HVA < 18 μmol/day.

3.3.2. Carcinoid tumor

A 64 yr, female patient had assay values for urine catecholic acids, after 1 in 4 dilution of urine, of HMMA = 16, 5-HIAA = 158 and HVA = 19 μmol/d (Fig. 4). The corresponding EQA median values

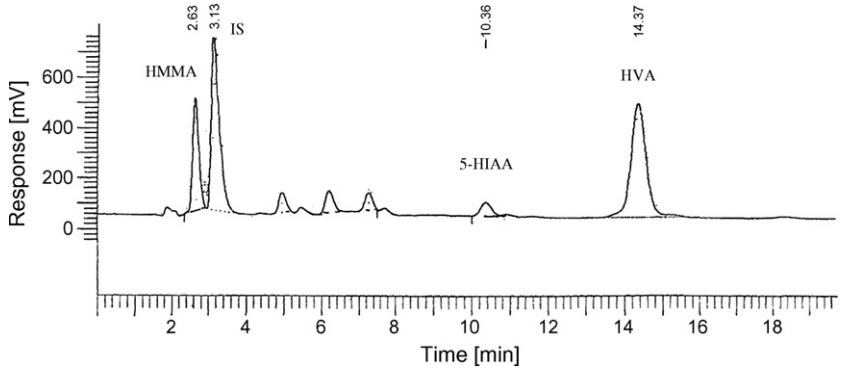

Fig. 3. Chromatogram of a patient diagnosed with neuroblastoma: HMMA (RT 2.6 min), IS (RT 3.1 min), 5-HIAA (RT 10.4 min), and HVA (RT 14.4 min).

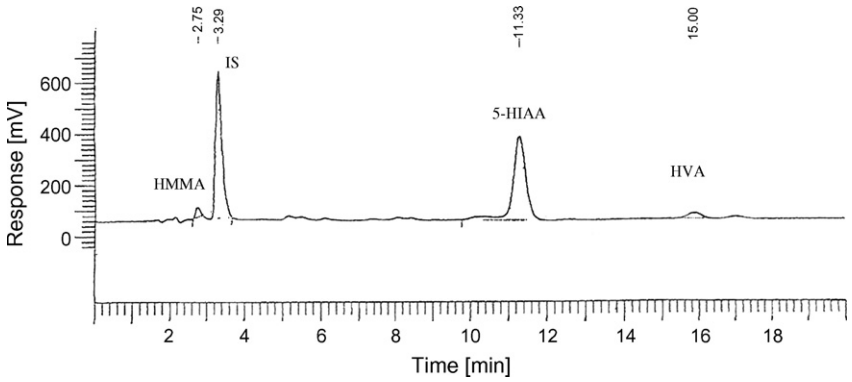

Fig. 4. Chromatogram of a patient diagnosed with carcinoid tumor: HMMA (RT 2.8 min), IS (RT 3.3 min), 5-HIAA (RT 11.3 min), and HVA (RT 15.9 min).

Table 3
Comparison of recovery (*R%*) and precision (COV%) data of current assay methods and this study for neuroblastoma and carcinoid tumor markers.

Method/detector	HMMA/VMA inter-day COV%	HMMA/VMA intra-day COV%	HMMA/VMA *R%*	HVA inter-day COV%	HVA intra-day COV%	HVA *R%*	5-HIAA inter-day COV%	5-HIAA intra-day COV%	5-HIAA *R%*	Ref.
GC/FID	4.4 (30.69) 9.3 (1.18)	5.2 (9.46)	No data	3.1 (9.46) 5.7 (1.04)	No data	No data	No data	No data	No data	[11]
HPLC/MS	No data	No data	89.69 (2.5) 92.64 (10) 102.17 (25)	No data	No data	95.63 (2.5) 100.13 (10) 100.22 (25)	No data	No data	81.16 (2.5) 91.03 (10) 99.24 (25)	[13]
SPE-HPLC/ECD	1.6, 2.4	5.9, 5.7	85	3.2, 4.3	5.9, 6.2	83	5.0, 3.5	9.2, 9.2	106	[12]
HPLC/ECD	9.5 (4.16)	15 (3.64)	93	7.1 (2.55)	18.7 (2.91)	98	No data	No data	No data	[14]
	4.9 (16.25)	6.3 (14.56)		5.0 (14.56)	7.5 (14.56)					
HPLC/ECD	4.1 (77.42)	4.9 (77.42)	97	5.3 (13.83)	11.6 (13.83)	97	5.8 (28.65)	7.1 (28.65)	90	[15]
HPLC/ECD	0.60 (3.31) 2.63 (14.85)	5.42 (3.29) 4.88 (16.24)	101.30	1.52 (2.40) 2.00 (18.2)	14.02 (1.95) 6.00 (18.2)	107.10	4.89 (3.51) 2.98 (26.93)	9.52 (3.21) 13.48 (26.93)	79.72	This study

Values in parenthesis = concentration (mg/L), CE: capillary electrophoresis; ECD: electrochemical detector; FID: flame ionization detector; GC: gas chromatography; HPLC: high pressure liquid chromatography; MS: mass spectrometry; Ref.: Reference; SPE: solid phase extraction; TMS: trimethylsilyl.

were 18, 165 and 16. The normal reference range is HMMA < 43, 5-HIAA < 50 and HVA < 42 μmol/day.

3.3.3. Pheochromocytoma

A 48-year male had typical urine catecholamines assay values 391, 821 and 28 μg/24 h for NE, *E* and DA, respectively. The reference range for hypertensive patients is NA < 104, *E* < 16, DA < 580 μg/24 h. The corresponding urine catecholic acids assay values were, after 1 in 4 dilution of urine, HMMA = 109, HVA = 38 and 5-HIAA = 28 μmol/day. The corresponding EQA median values were 119, 30 and 31. The normal reference range is HMMA < 43, HVA < 42, 5-HIAA < 50 μmol/day.

3.4. Comparison with previous and current methods

Whilst various methods have been reported for assay of these target analytes, they are not without some level of disadvantage. Our goal was to develop a simple, cost-effective procedure as regards the analytical equipment and the actual assay that would be able to accurately quantitate all three target analytes in one run.

The capillary GC–FID procedure [11] involves prior derivatisation with BSTFA. Reported recoveries were inconsistently between 40% and 90% for the target analytes. The method does not report assay of 5-HIAA (Table 3) and no proficiency testing data was reported.

For solid phase extraction methods, with HPLC-electrochemical detection [12], the recoveries ranged from 83% to 106% with internal standard recovery of 91%. No proficiency testing data to ascertain accuracy was reported.

Although the use of a mass spectrometric detector, coupled to GC or HPLC, is selective and specific, the cost implications preclude its widespread use. The recent HPLC–MSMS [13] method reported recoveries of 90–102% for HMMA and HVA, but slightly lower recovery was observed for 5-HIAA, ranging from 81 to 99% over the range 2.5–25 mg/L. The CV% for recovery at the spike concentration of 10 mg/L was relatively high at 16.88%. No precision data for the assay, for any of the three analytes, was reported (Table 3).

None of the reported methods [14,15] have extensive validation data comparable to the present study, except the HPLC-ECD procedure, by Davidson [15]. However, there is no data on any LOD levels for the same target analytes. Our recovery and precision data compares fairly well with his results for both HMMA and HVA, with similarly poorer day-to-day precision (14% [15] and 2, 18% (this study)) obtained for HVA. For 5-HIAA, similarly reduced recovery of 80% and 90%, and day-to-day precision of 7% and 13%, obtained by Davidson and this study, respectively, is also comparable (Table 3). Although Davidson reports proficiency testing results for both HMMA and HVA, no data is reported for 5-HIAA, as is the case with this present study. Comparison with the HPLC-mass spectrometric detection method [13] indicates that no proficiency testing data has been reported for any of the three target analytes.

4. Conclusion

An HPLC method using electrochemical detection was developed for routine assay of urine catecholic acids (HMMA, HVA) and 5-HIAA, simultaneously. The new method was shown to be accurate, precise, simple and rapid and compared fairly well with similar, current methods.

The cost benefits, use of relatively non-toxic chemicals and reagents, and ease of use of the present assay makes it an attractive option to the more sophisticated methods currently reported. The assay was successfully applied for diagnosis of patients with various neoplastic diseases.

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