*Journal of Chromatography*, 487 (1989) 29–39
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4499

# ANALYSIS OF THE O-METHYLATED METABOLITES OF ISOPRENALINE, ADRENALINE AND NORADRENALINE IN PHYSIOLOGICAL SALT SOLUTIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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(First received May 6th, 1988; revised manuscript received September 16th, 1988)

## SUMMARY

This study provides the first report of a sensitive, simple and rapid high-performance liquid chromatographic (HPLC) assay for the simultaneous analysis of isoprenaline and its metabolite, 3-O-methylisoprenaline, in samples of physiological salt solutions. The assay does not require time-consuming sample clean-up or extraction procedures and uses a Nova-Pak $C_{18}$ column, an isocratic mobile phase and an amperometric detector. In addition, small modifications to the composition of the mobile phase have also provided sensitive assays for noradrenaline and adrenaline and their O-methylated or O-methylated deaminated metabolites (normetanephrine, metanephrine, 3-methoxy-4-hydroxyphenylethylene glycol and 3-methoxy-4-hydroxymandelic acid) These HPLC assays are sufficiently sensitive and rapid to replace the use of [$^3$H]amines and column chromatographic separation of the metabolites for most in vitro studies on the uptake and subsequent metabolism of catecholamines by monoamine oxidase and/or catechol-O-methyltransferase in tissues.

## INTRODUCTION

Noradrenaline, adrenaline and isoprenaline are the most commonly used substrates for studies on the uptake and metabolism of catecholamines in tissues in vitro. Extraneuronal uptake and subsequent O-methylation of catecholamines in tissues have been studied with isoprenaline as the substrate amine, e.g. in vascular [1–4] and non-vascular [5–7] smooth muscle and in the myocardium [8–10], and noradrenaline and adrenaline have also been used in studies on the myocardium [11–13] and vascular smooth muscle [14]. Most of these studies have used tritiated amines and this has involved the separation of the parent amine from the metabolites by column chromatography followed by liquid scintillation

0378-4347/89/$03.50 

counting of each fraction. One disadvantage of using radiolabelled amines is that [$^3$H]adrenaline and [$^3$H]isoprenaline are not available commercially as a suitable form of the (−)-isomer, for which extraneuronal uptake is stereoselective [13,15,16]. Unlabelled amines, in the appropriate isomeric form, can be used if fluorescence microphotometry is used to measure the amine uptake in situ in tissues [5,15,17], but this technique cannot be used for studies on the metabolism of the amines, because the metabolites do not form formaldehyde-induced fluorophores [5,18].

High-performance liquid chromatography (HPLC) with electrochemical detection (ED) offers the advantages that the unlabelled amine in the desired isomeric form can be used and the parent amine and its metabolites can be separated and measured. The only report in the literature of an HPLC assay for isoprenaline and its metabolite, 3-O-methylisoprenaline (OMI), involves time-consuming separation of these two compounds prior to injecting them onto the HPLC column [19]. HPLC assays for noradrenaline and adrenaline have analyzed only one or two of the metabolites (e.g. ref 20), have involved a time-consuming separation of the metabolites [21] or have required the use of either a flow gradient [22] or a solvent gradient and a change of electrode potential [23] during the assay. The aim of the present study was to develop an HPLC assay for isoprenaline, adrenaline and noradrenaline and their O-methylated metabolites in physiological salt solutions, using an isocratic mobile phase and minimal preparation prior to injection of the sample onto the column.

## EXPERIMENTAL

### *Apparatus*

The HPLC apparatus consisted of a Waters Assoc. (Millipore, Newstead, Brisbane, Australia) Model 510 pump and solvent delivery system, a Waters U6K universal injector, a $C_{18}$ or CN (see Results) Guard-Pak precolumn module (Millipore) and a Nova-Pak $C_{18}$ stainless-steel column (15 cm × 3.9 mm I.D. with 5-$\mu$m bonded packing; Millipore). A Waters Assoc. Model 410 amperometric electrochemical detector with a glassy carbon working electrode and Ag/AgCl reference electrode were used. The working electrode potential was varied during the experiments (see Results). Peak heights, recorded on a BBC SE120 chart recorder (Millipore), were used to quantify the results.

### *Mobile phase*

The mobile phase developed for the assay of the different amines and their metabolites (see Results) were all based on a 70 m*M* $Na_2HPO_4$ solution prepared in triple distilled water. The final solution was adjusted to pH 3.6 with 8.7 *M* phosphoric acid and was filtred through a Millipore FH 0.5-$\mu$m filter under vacuum and then degassed under vacuum in a sonicator for 4 min. The mobile phase was filtered and degassed each morning and was recirculated through the HPLC system for one week. An optimal flow-rate was established for each assay (see Results) and when the HPLC was not in use for an assay the mobile phase was recirculated at 0.1 ml/min. Recirculation of the mobile phase markedly improves

the stability of the electrochemical detector and it also allows replacement of silica stripped from the column packing by the ionpairing agent, sodium heptanesulphonate (HSA) [24].

*Physiological salt solutions*

Experiments were carried out to test the stability of the amines and their metabolites under the conditions that would occur in experiments with tissues. The physiological salt solutions used were Krebs solution containing 118 m*M* NaCl, 4.7 m*M* KCl, 2.5 m*M* $CaCl_2 \cdot 2H_2O$, 1.2 m*M* $KH_2PO_4$, 1.2 m*M* $MgSO_4 \cdot 7H_2O$, 11.7 m*M* glucose and 0.04 m*M* $Na_2EDTA$, and Tyrode solution containing 137.3 m*M* NaCl, 2.3 m*M* KCl, 1.3 m*M* $CaCl_2 \cdot 2H_2O$, 0.4 m*M* $KH_2PO_4$, 1.05 m*M* $MgCl_2 \cdot 6H_2O$, 11.9 m*M* $NaHCO_3$, 5.0 m*M* glucose and 0.04 m*M* $Na_2EDTA$. The solutions were aerated with oxygen–carbon dioxide (95:5) and incubated at 37°C for specified times after addition of the amines and/or their metabolites where indicated (see Results). For HPLC analysis of samples in physiological salt solutions, samples (1 ml) were taken, and 100 $\mu$l of ice-cold 5 *M* acetic acid and 10 $\mu$l of 0.27 *M* $Na_2EDTA$ were added immediately. The samples were then stirred and immediately frozen until they were required for injection onto the HPLC column.

In experiments on O-methylation of isoprenaline in guinea pig trachea, segments of trachea were incubated in 2 ml of 0.1 or 1 $\mu M$ (±)-[$^3$H]isoprenaline in Krebs solution aerated with oxygen–carbon dioxide (95:5) at 37°C for 40 min. The OMI in the incubation solution at the end of the period of incubation with isoprenaline was analyzed both by HPLC (see above) and by column chromatography and liquid scintillation counting as described by Bryan et al. [17].

*Standards*

External standards were prepared in triplicate with a range of concentrations of each of the compounds in the particular HPLC assay. The standards were prepared just prior to commencement of the assay in the same solvent as the samples for analysis and were treated as for the samples, e.g. addition of acetic acid and EDTA. The standards were injected onto the column several times at the beginning of the assay and once after every three samples during the assay (i.e. every 30–45 min depending on the analysis time per sample). The intra-assay coefficients of variation of the ED current responses for the standards of each of the compounds were 1.5–4%. These low values indicated that loop column losses were consistent from sample to sample and there was no marked change in ED sensitivity during the assay period of a working day.

*Drugs and solutions*

The drugs used in the study were: (−)-adrenaline bitartrate (Sigma, St. Louis, MO, U.S.A.); (±)-3,4-dihydroxymandelic acid (DOMA; Sigma); (±)-3,4-dihydroxyphenylethylene glycol (DOPEG, Sigma); (±)-isoprenaline sulphate (Sigma); (±)-[7-$^3$H]isoprenaline (Amersham International, Amersham, U.K., 500 MBq/$\mu$mol, purified over alumina before use and diluted with unlabelled (±)-isoprenaline to the desired final specific activity); (±)-metanephrine hy-

drochloride (Calbiochem-Behring, La Jolla, CA, U.S.A.); 3-methoxy-4-hydroxymandelic acid (VMA; Sigma); 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG; Sigma); 3-O-methylisoprenaline hydrochloride (OMI·HCl; Boehringer-Ingelheim, Ingelheim, F.R.G.); (−)-noradrenaline bitartrate (Sigma); (±)-normetanephrine hydrochloride (Sigma).

Drug stock solutions (10 m*M*) were prepared in 10 m*M* hydrochloric acid and were also diluted with 10 m*M* hydrochloric acid unless otherwise indicated (e.g. when Krebs or Tyrode solutions were used, see Results).

## RESULTS

### *HPLC analysis of isoprenaline and OMI*

The composition of the mobile phase that gave the best separation of isoprenaline and OMI both from the solvent front and from each other was: 70 m*M* $Na_2HPO_4$, 5 m*M* sodium HSA, 0.1 m*M* $Na_2EDTA$ and 9% (v/v) acetonitrile, adjusted to pH 3.6, at a flow-rate of 1.0 ml/min. The retention times of isoprenaline and OMI under these conditions were 4.2 and 8.0 min, respectively. Over a period of two years, the retention times varied by ±0.2 min from these values, but they were very consistent on a particular day because the column was insulated from temperature fluctuations by polystyrene foam. For specific applications (e.g. if there was a broad solvent peak interfering with the isoprenaline peak), it would be possible to increase the retention times by decreasing the acetonitrile content of the mobile phase.

The hydrodynamic voltammogram (HDV) for isoprenaline reached a maximum at a working electrode potential of 0.825 V versus Ag/AgCl and that for OMI increased only slightly when the electrode potential was increased from 0.825 to 0.850 V (Fig. 1). The background noise also increased at the higher electrode potentials, so 0.825 V was selected for all subsequent analyses of isoprenaline and OMI.

The relationship between ED current response and amounts of amine injected onto the column was linear for isoprenaline (0.1–240 pmol) and for OMI (0.5–240 pmol). The equations of the regression lines were

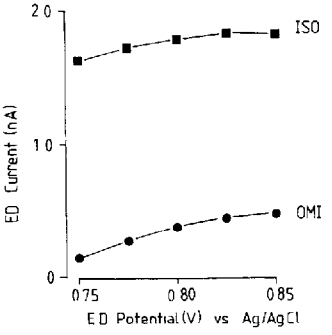


Fig. 1. Hydrodynamic voltammograms for isoprenaline (ISO) and 3-O-methylisoprenaline (OMI). Injections of 5 pmol of each compound were made. A $C_{18}$ guard column was used. See Results for details of mobile phase composition, etc.

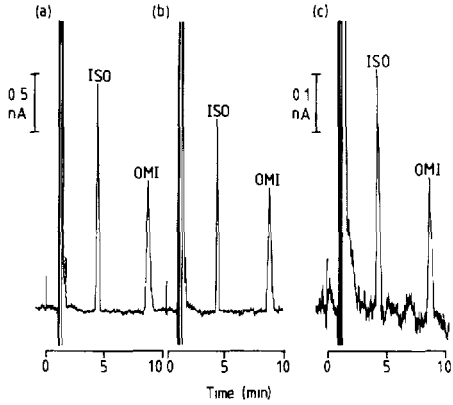


Fig. 2. Chromatograms of mixtures of 5 pmol (a and b) or 1 pmol (c) of each of isoprenaline (ISO) and 3-O-methylisoprenaline (OMI), prepared in Krebs solution and with acetic acid and $Na_2EDTA$ present to stabilize the amines (see Experimental). The sample in (b) was taken from a solution that had been aerated with oxygen–carbon dioxide (95 5) and incubated at 37°C for 60 min, prior to addition of the acetic acid and $Na_2EDTA$. A CN guard column was used. See Results for details of mobile phase composition, etc.

$y=(0.366\pm0.004)x+(0.006\pm0.007)$, $r=0.9991$, $n=21$ for isoprenaline and $y=(0.090\pm0.001)x+(0.013\pm0.003)$, $r=0.9992$, $n=9$ for OMI. Hence, the response for isoprenaline was 0.366 nA/pmol and for OMI was 0.090 nA/pmol, when a $C_{18}$ Guard-Pak was in use. However, when the Guard-Pak was changed to a CN column, the response for OMI increased to 0.25 nA/pmol (Fig. 2) and the background noise decreased, but there was no change in the response for isoprenaline. A CN guard column was used for all subsequent analyses and under these conditions the limit of detection at a signal-to-noise ratio of 2.0 was 0.1 pmol for isoprenaline and 0.2 pmol for OMI. The intra-assay coefficients of variation were 5.7–7.1% for 0.1–0.5 pmol isoprenaline, 1.0–4.1% for 1.0–240 pmol isoprenaline and 1.0–4.3% for 0.5–240 pmol OMI ($n=4$–6 for each amount of isoprenaline or OMI). The inter-assay coefficients of variation were 9.9% ($n=9$) and 7.0% ($n=8$), respectively, for 0.5 and 5.0 pmol isoprenaline and 5.6% ($n=8$) and 5.1% ($n=8$), respectively, for 0.5 and 5.0 pmol OMI.

*Application of HPLC analysis of isoprenaline and OMI to samples in physiological salt solutions*

It was necessary to omit ascorbic acid from the physiological salt solutions for HPLC analysis of the amines to avoid a very large increase in ED current which lasted several hours at the concentration of 0.57 *M* that is routinely included in the salt solutions when other methods of sample analysis are used. EDTA was still present to prevent decomposition of the amines and their metabolites in the physiological salt solutions.

Samples of isoprenaline and OMI in either Krebs solution (Fig. 2a and c) or Tyrode solution gave the same retention times and ED current responses as did samples in 10 m*M* hydrochloric acid. There was still good separation of the isoprenaline and OMI from the solvent peak. The intra-assay coefficients of varia-

tion were 5.3 and 2.4%, respectively, for 0.5 and 5.0 pmol isoprenaline and 4.3 and 2.8%, respectively, for 0.5 and 5.0 pmol OMI in Krebs solution and corresponding values for samples in Tyrode solution were 2.8 and 1.9% and 4.7 and 1.0%, respectively ($n=5$–7). The inter-assay coefficients of variation were 6.6 and 5.7%, respectively, for 0.5 and 5.0 pmol isoprenaline and 4.5 and 5.3%, respectively, for 0.5 and 5.0 pmol OMI in Krebs solution and corresponding values for samples in Tyrode solution were 4.2 and 3.1% and 7.8 and 4.5%, respectively ($n=8$–9).

In order to simulate conditions in incubation experiments in which tissues are incubated in isoprenaline, samples of isoprenaline and OMI in Krebs solution (Fig. 2b) or in Tyrode solution were aerated with oxygen–carbon dioxide (95:5) and incubated at 37 °C for 60 min. Ice-cold EDTA and acetic acid were immediately added to samples of the solution at the end of the incubation period (see Experimental). Samples were analyzed either on the same day or after three days' storage in the freezer at −20 °C to determine whether isoprenaline and OMI samples were sufficiently stable to allow storage of the samples for several days after an experiment. After incubation and analysis on the same day, OMI at concentrations of 0.01–1 $\mu M$ was stable. There was no loss of isoprenaline at a concentration of 1 $\mu M$, but a loss of up to 26% at concentrations of 0.1 and 0.01 $\mu M$ isoprenaline. Storage of the samples in the freezer at −20 °C for three days resulted in no decomposition of OMI, but some additional loss of isoprenaline. In subsequent experiments, samples were routinely analyzed on the day of collection.

The application of the HPLC analysis to experiments with tissues was further examined by measuring OMI in the incubation solution, after segments of guinea pig trachea were incubated in [$^3$H]isoprenaline (see Experimental for details).

TABLE I

COMPARISON OF ASSAY OF OMI IN THE INCUBATION SOLUTION BY HPLC-ED AND LIQUID SCINTILLATION COUNTING OF $^3$H, AFTER EXPOSURE OF GUINEA PIG TRACHEAL SEGMENTS TO ISOPRENALINE

Tracheal segments from guinea pigs were incubated in 2 ml of 0.1 $\mu M$ ($n=2$) or 1 $\mu M$ ($n=3$) (±)-[$^3$H]isoprenaline at 37 °C for 40 min. The OMI in samples of each incubation solution were assayed by (a) HPLC (see Experimental for details), using 5- or 50-$\mu$l injection volumes or (b) separation of isoprenaline and OMI by column chromatography and liquid scintillation counting of $^3$H in the separated fractions [17]. The assays of OMI in the samples were then calculated as OMI production by the tissues in pmol/g. The relative standard deviations for the results obtained with these two methods were 3.2% at 0.1 $\mu M$ isoprenaline and 6.9% at 1 $\mu M$ isoprenaline.

| Isoprenaline concentration ($\mu M$) | OMI production (pmol/g) | |
|---|---|---|
| | HPLC-ED | $^3$H |
| 0.1 | 134 | 131 |
| 0.1 | 129 | 124 |
| 1.0 | 1312 | 1122 |
| 1.0 | 929 | 846 |
| 1.0 | 1081 | 947 |

The results (Table I) show that there was very close correlation ($r=0.992, n=5$) between the OMI in the samples measured using the HPLC assay and that using column chromatography and liquid scintillation counting, and the relative standard deviations for the results obtained with the two methods were low (Table I).

*HPLC analysis of noradrenaline and adrenaline and metabolites*

The composition of the the mobile phase that gave the best separation of the O-methylated metabolites of noradrenaline and adrenaline (Fig. 3) was: 70 m*M* $Na_2HPO_4$, 1 m*M* sodium HSA and 0.1 m*M* $Na_2EDTA$, adjusted to pH 3.6, at a flow-rate of 0.7 ml/min. The retention times of the O-methylated metabolites under these conditions were 3.6 min (VMA), 5.0 min (MOPEG), 7.2 min (normetanephrine) and 15.0 min (metanephrine). The concentration of the ion-pairing agent, HSA, was critical in that lower concentrations resulted in overlap of the peaks for MOPEG (unaffected by HSA) and normetanephrine (retention time increased by HSA). On the other hand, higher concentrations of HSA increased the assay time by increasing the retention time of metanephrine.

The HDV was at a maximum for normetanephrine, metanephrine and MOPEG over the range of working electrode potentials tested (0.775–0.850 V versus Ag/AgCl), but required a potential of 0.825 V versus Ag/AgCl to reach a maximum for VMA (Fig. 4). Hence, a potential of 0.825 V versus Ag/AgCl was selected for subsequent analyses of these O-methylated metabolites. Under these conditions, the ED current responses were 0.60 nA/pmol for VMA, 0.43 nA/pmol for normetanephrine, 0.22 nA/pmol for metanephrine and 0.15 nA/pmol for MOPEG. The limits of detection, defined as the amount of compound that gives

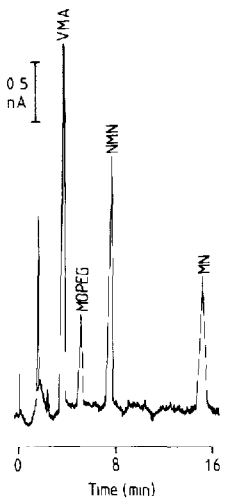


Fig. 3. Chromatogram of mixtures of 5 pmol of each of 3-methoxy-4-hydroxymandelic acid (VMA), 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG), normetanephrine (NMN) and metanephrine (MN), prepared in 0.01 *M* hydrochloric acid A CN guard column was used. See Results for details of mobile phase composition, etc.

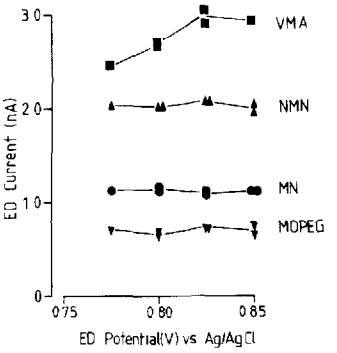


Fig. 4. Hydrodynamic voltammograms for 3-hydroxy-4-methoxymandelic acid (VMA), normetanephrine (NMN), metanephrine (MN) and 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG). Injections of 5 pmol of each compound were made. A CN guard column was used. See Results for details of mobile phase composition, etc.

a signal-to-noise ratio of 2.0, were 0.1 pmol for VMA and normetanephrine, 0.2 pmol for metanephrine and 0.3 pmol for MOPEG. The intra-assay coefficients of variation were 3.8, 1.4, 1.3 and 2.5% for VMA, normetanephrine, metanephrine and MOPEG, respectively ($n=4$), and the inter-assay coefficients of variation were 5.2, 3.0, 4.1 and 5.0%, respectively, for the corresponding metabolites ($n=6$).

HPLC analysis of the parent amines, noradrenaline and adrenaline, could be readily achieved with a mobile phase of the composition used to analyze isoprenaline and OMI provided that the acetonitrile content was reduced. The composition of the mobile phase was 70 m*M* $Na_2HPO_4$, 5 m*M* sodium HSA, 0.1 m*M* $Na_2EDTA$ and 2% (v/v) acetonitrile, adjusted to pH 3.6, at a flow-rate of 1.0 ml/min and an ED potential of 0.825 V versus Ag/AgCl. Under these conditions the retention times and ED current responses, respectively, were 3.2 min and 0.374 nA/pmol for noradrenaline and 5.0 min and 0.300 nA/pmol for adrenaline. The intra-assay coefficients of variation were 2.8 and 5.2% for noradrenaline and adrenaline, respectively ($n=4$), and the inter-assay coefficients of variation were 4.0 and 6.5%, respectively, for the corresponding amines ($n=6$).

The analysis of the deaminated metabolites, DOMA and DOPEG, proved to be impossible with the Nova-Pak $C_{18}$ column. Various changes in the mobile phase, e.g. changes in pH, flow-rate and the concentrations of acetonitrile, sodium HSA and $Na_2HPO_4$, all failed to separate DOMA and DOPEG from the solvent front.

## DISCUSSION

The principal aim of this study was to develop an HPLC assay for isoprenaline and OMI in samples of physiological salt solutions for application to studies on extraneuronal uptake and O-methylation of catecholamines. [$^3$H]Amines are required for in vivo studies on clearance and turnover of catecholamines. However, for in vitro studies in tissues, an HPLC assay for isoprenaline and OMI would allow isoprenaline stereoisomers (not available in labelled form) to be used and the possibility of an isotope effect of labelled amines [25] would not have to be considered.

Previous HPLC assays reported for OMI have involved extraction of OMI either from samples of physiological salt solutions [19] or from urine and plasma samples [26] prior to injection onto the column. The HPLC assay developed in this study for samples in physiological salt solutions involves no preliminary extraction of isoprenaline and OMI and hence uses the chromatographic properties of the column to separate the amine and its metabolite. Samples can be injected at 10-min intervals, so that samples can be rapidly analyzed on the day they are collected. The electrode potential that was selected as optimal for the assay (0.825 V versus Ag/AgCl) was very close to the potential of 0.80 V versus Ag/AgCl used in previous assays for OMI [19,26]. The assay is very sensitive with ED current responses about 6.5 times greater for isoprenaline and 4.7 times greater for OMI than those of Barone et al. [19]. The lower sensitivity of the latter assay [19] is partly due to losses during extraction of isoprenaline and OMI, but additional factors must be involved, such as lower sensitivity of the electrochemical detector, lower efficiency of the column, etc. It may be possible (i) to further improve the sensitivity of the HPLC assay by the use of microbore (1.2 mm I.D.) columns [27,28] and (ii) to avoid loss of sensitivity by surface passivation of the working electrode over a period of several months by use of the recently introduced E.C. Monitor tubular electrode [29]. Sulphate conjugates of isoprenaline and OMI in tissues could also be assayed in tissues by HPLC assay of samples before and after acid hydrolysis to release the unconjugated amines [30].

The analysis by HPLC of the metabolites of noradrenaline or adrenaline is a much more complex problem than that of isoprenaline and OMI. It is almost impossible to devise one HPLC assay using an isocratic mobile phase for the five metabolites and the parent amine, because of the presence of bases, acids and neutral metabolites of very different polarities. Hence, most reported HPLC assays have measured only one or two of the metabolites of noradrenaline or adrenaline, e.g. DOMA and DOPEG [20], normetanephrine [31,32], metanephrine [33], MOPEG [33–35] or VMA [36].

An assay has been reported [21] for noradrenaline and its five metabolites by an initial alumina separation into the catechol fraction (noradrenaline, DOPEG and DOMA) and the non-catechol fraction (normetanephrine, MOPEG and VMA) and HPLC analysis of each of these two fractions. However, the ED current responses were very low for all of the compounds (0.045–0.1 nA/pmol) [21]. In the present study, an assay using an isocratic mobile phase was developed for the non-catechol metabolites (normetanephrine, metanephrine, MOPEG and VMA), with ED current responses two- to ten-fold greater than those reported by Oishi et al. [21]. The increased sensitivity in the present study was partly due to the lack of a complex extraction procedure, but also must be due to higher ED sensitivity, higher column efficiency, etc. The catecholamines, noradrenaline and adrenaline, could be assayed using a different mobile phase, but it was not possible with the Nova-Pak $C_{18}$ column to find a system that would also separate DOPEG and DOMA from the solvent front. Possible solutions for the development of a suitable assay for these metabolites would be to use a column with greater retention properties or to develop a sample derivatisation procedure.

In conclusion, a very sensitive, simple and rapid HPLC–ED assay has been

developed for the simultaneous analysis of isoprenaline and OMI in samples of physiological salt solutions, with no prior sample clean-up or extraction procedures and with an isocratic mobile phase. In addition, small modifications to the composition of the mobile phase have also provided sensitive assays for noradrenaline and adrenaline and their O-methylated metabolites (normetanephrine, metanephrine, MOPEG and VMA), but more significant changes to the HPLC system are required to analyze DOPEG and DOMA.

ACKNOWLEDGEMENTS

We would like to thank Helen Bovill and Kathy Lovell for excellent technical assistance. The support of this work by a grant from the National Health and Medical Research Council of Australia is also gratefully acknowledged.

REFERENCES

1 R.J. Head, I.S. de la Lande, R.J. Irvine and S.M. Johnson, Blood Vessels, 17 (1980) 229.
2 D. Branco, I. Azevedo, A. Sarmento and W. Osswald, Naunyn-Schmiedeberg's Arch. Pharmacol., 316 (1981) 120.
3 L.J. Bryan, S.R. O'Donnell and U. Trendelenburg, Naunyn-Schmiedeberg's Arch. Pharmacol., 328 (1984) 56.
4 S. Barone, R.E. Stitzel and R.J. Head, Naunyn-Schmiedeberg's Arch. Pharmacol., 329 (1985) 9.
5 E.N. Anning, L.J. Bryan and S.R. O'Donnell, Br. J. Pharmacol., 65 (1979) 175.
6 L.G. Garland, N.V. Marrion and G.R. Martin, Naunyn-Schmiedeberg's Arch. Pharmacol., 318 (1981) 88.
7 E.N. Anning, S.R. O'Donnell and J.C. Wanstall, Br. J. Pharmacol., 62 (1978) 472P.
8 H. Bönisch, Naunyn-Schmiederberg's Arch. Pharmacol., 303 (1978) 121.
9 L.J. Bryan, H. Fleig and U. Trendelenburg, Naunyn-Schmiedeberg's Arch. Pharmacol., 322 (1983) 6.
10 U. Trendelenburg, H. Fleig, L.J. Bryan and H. Bönisch, Naunyn-Schmiedeberg's Arch. Pharmacol., 324 (1983) 169.
11 E.R. Fiebig and U. Trendelenburg, Naunyn-Schmiedeberg's Arch. Pharmacol., 303 (1978) 21.
12 E.R. Fiebig and U. Trendelenburg, Naunyn-Schmiedeberg's Arch. Pharmacol., 303 (1978) 37.
13 M Grohmann and U. Trendelenburg, Naunyn-Schmiedeberg's Arch. Pharmacol., 328 (1984) 164.
14 M. Henseling, Naunyn-Schmiedeberg's Arch. Pharmacol., 323 (1983) 12.
15 L.J. Bryan and S.R. O'Donnell, Br. J. Pharmacol., 82 (1984) 757
16 M. Henseling, Naunyn-Schmiedeberg's Arch. Pharmacol , 328 (1984) 219.
17 L.J. Bryan, S.R. O'Donnell and C.F. Wildsoet, Naunyn-Schmiedeberg's Arch. Pharmacol., 327 (1984) 133.
18 H. Corrodi and G. Johnson, J. Histochem. Cytochem., 15 (1967) 65.
19 S. Barone, R.E. Stitzel and R.J. Head, J. Chromatogr., 310 (1984) 283.
20 K. Starke, L. Hedler and A. Steppeler, Naunyn-Schmiedeberg's Arch. Pharmacol., 317 (1981) 193.
21 R. Oishi, S. Mishima and H. Kuriyama, Life Sci., 32 (1983) 933.
22 Z.L. Rossetti, G. Mercuro and C.A. Rivano, Life Sci., 33 (1983) 2387.
23 T. Ishimitsu and S. Hirose, J. Chromatogr , 337 (1985) 239.
24 P.J. Brent, S. Hall, A.J Smith and J. Aylward, J. Pharmacol. Methods, 14 (1985) 243.
25 U. Trendelenburg, F.J.E. Stefano and M. Grohmann, Naunyn-Schmiedeberg's Arch. Pharmacol., 323 (1983) 128.
26 R.C. Causon, M.J. Brown and D.S. Davies, J. Chromatogr., 337 (1985) 311.

27 T.A. Durkin, E.J. Caliguri, I.N. Mefford, D.M. Lake, I.A. Macdonald, E. Sundstrom and G. Jonsson, Life Sci., 37 (1985) 1803.
28 G. Kamperman and J.C. Kraak, J. Chromatogr., 337 (1985) 384.
29 J.B. Nair, M.N. Munk and J.D. McLean, J. Chromatogr., 416 (1987) 340.
30 R.C. Causon, R. Desjardins, M.J. Brown and D.S. Davies, J. Chromatogr., 306 (1984) 257.
31 D.T. Borchardt, M.F. Hegazi and R.L. Schowen, J. Chromatogr., 152 (1978) 255.
32 J. Jouve, N. Mariotte, C. Sureau and J.P. Muh, J. Chromatogr., 274 (1983) 53.
33 G.L. Diggory and W.R. Buckett, J. Pharmacol. Methods, 11 (1984) 207.
34 R.F. Seegal, K.O. Brosch and B. Bush, J. Chromatogr., 377 (1986) 131.
35 N.S. Sharpless, U. Halbreich and H. Feldfogel, J. Chromatogr., 377 (1986) 101.
36 E. Gerlo and R. Malfait, J. Chromatogr., 343 (1985) 9.