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ANALYSIS OF CYSTEINYLDOPAS, DOPA, DOPAMINE, NORADRENALINE AND ADRENALINE IN SERUM AND URINE USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ELECTROCHEMICAL DETECTION

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SUMMARY

The catecholic amino acids, dopa, 2-S- and 5-S-cysteinyldopa, and 2,5-S,S-dicysteinyl-dopa were determined qualitatively in serum from patients with malignant melanoma by reversed-phase high-performance liquid chromatography, using electrochemical detection. In urine the catecholamines dopamine, noradrenaline and adrenaline were also determined qualitatively, as well as the above-mentioned compounds, in a single chromatographic run. The conditions were optimized by changing the pH of the mobile phase and by the addition of methanesulphonic acid. A comparison was made between the performance of four commercial reversed-phase packing materials containing chemically bonded octadecyl groups, using a standard mixture of catecholic amino acids. The influence of ionic strength, pH and amount of methanesulphonic acid on retention was investigated.

INTRODUCTION

5-S-Cysteinyldopa is a newly discovered amino acid occurring in both normal subjects and, in increased amounts, in patients with malignant melanoma. Its concentration in the urine has been determined to obtain information on the degree of dissemination of the tumour. The fluorometric method [1] used for this determination is sensitive enough to detect this amino acid in serum

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only in advanced cases. The separation and quantitative analysis of this compound and dopa in serum by liquid chromatography was recently described by the present authors [2]. An amperometric detector according to Kissinger [3] was used which permitted detection of very small amounts, i.e. 25 pg, of each compound. The separation was performed on a Nucleosil C_{18} phase and the mobile phase consisted of 0.5% (v/v) methanol in water, containing 2.9 g of phosphoric acid per litre. This packing material contains chemically bonded octadecyl groups on microparticulate silica.

A useful means of varying the retention in such reversed-phase systems was described by Knox and Jurand [4], who added anionic detergents to the mobile phase. This method was named "soap chromatography", and applied to the separation of catecholamines and their metabolites. The retention mechanism in this kind of chromatography has recently been discussed [5, 6]. Another example of the utility of chemically bonded reversed-phase packing materials for the analysis of catecholamines and related substances is given by Molnár and Horváth [7], who separated acidic and basic catecholamine metabolites by varying the pH and ionic strength of the mobile phase. High-performance ion-exchange chromatography has also been shown to be useful for the analysis of dopa and catecholamines, i.e. dopamine, noradrenaline and adrenaline [8, 9], in therapeutic serum and urine (see Fig. 1).
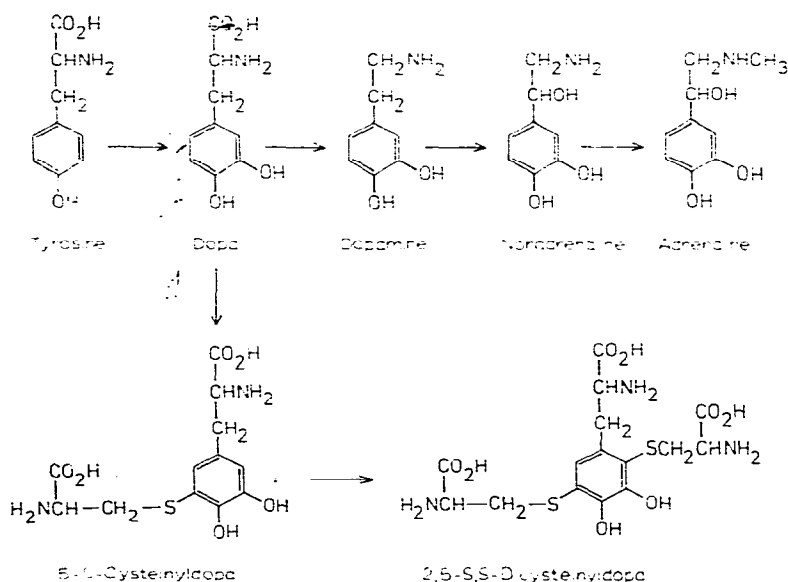

Fig. 1. The biochemical pathways for the formation of cysteinyldopas and catecholamines from tyrosine.

As the concentration of the actual catecholic compounds is generally low in biological fluids, detection is often a problem. In many instances neither UV nor fluorescence detectors can be used, and although increased sensitivity can be obtained by derivatization [10], the method is often less attractive due to incomplete reaction. In this situation the electrochemical detector developed

by Kissinger [3], which, as already mentioned, was used in our previous work on 5-S-cysteinyldopa and dopa, has satisfied a long-felt need in the case of easily oxidizable substances.

5-S-Cysteinyldopa is the major cysteinyldopa in serum and urine. In addition to this compound, three other cysteinyldopas, namely 2-S- and 6-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa, have been identified in urine [11, 12]. The concentrations of all these amino acids in biological fluids are of great interest, since they reflect the oxidation status in the melanocyte. In this work, the separation and sensitivity of determination of cysteinyldopa and other tyrosine metabolites such as dopa, dopamine, adrenaline and noradrenaline in serum and urine have been studied in order to achieve the simultaneous analysis of these compounds (see Fig. 1). To this end, a thorough study of the utility of various commercial reversed-phase packing materials of the C_{18} type was made and the properties of the mobile phase changed by varying its pH and ionic strength. The effect of adding anionic modifiers was also investigated. Electrochemical detection was used throughout, except in the study of the effect of adding sodium sulphate to the mobile phase, where a UV detector was used.

The variation of sensitivity with the working potential of the electrochemical detector was studied for some of the compounds. The compatibility of the detector with the various mobile phases was also examined. A column packing technique especially suited to reversed-phase packing material is described in detail. As in our previous investigation, alumina was used to prepurify samples of urine and serum. The recovery of catecholic amino acids after elution with acids of different strengths was established. The present study is mainly concerned with the development of proper separation conditions for the compounds of interest.

EXPERIMENTAL

Apparatus
A Varian Model 8500 (Varian, Palo Alto, Calif., U.S.A.) positive displacement pump was used. Samples were injected with a valve injector Rheodyne model 7120 (Rheodyne, Berkeley, Calif., U.S.A.).

In most cases a thin-layer amperometric detector, Model LC-10 (Bioanalytical Systems, West Lafayette, Ind., U.S.A.) was used. The electrode was in most cases operated at 0.75 V vs. an Ag—AgCl reference electrode. The graphite paste was CPO.

A variable wavelength UV detector, Varian Vari-Chrom liquid chromatography detector, was used for studying the effect of adding sodium sulphate to the mobile phase.

Column packing material
Four different commercially available reversed-phase packing materials were used. The mean particle diameter was 10 μm unless otherwise stated: Nucleosil C_{18} (5 or 10 μm) (Macherey, Nagel & Co., Düren, G.F.R.), Spherisorb ODS (Phase Separations, Queensferry, Great Britain), LiChrosorb RP-18 (E. Merck, Darmstadt, G.F.R.), Partisil ODS (Whatman, Clifton, N.J., U.S.A.).

Column tubing and fittings

The columns consisted of 200 × 6.35 mm O.D. × 5 mm I.D. stainless-steel 316 tubing with either a polished or unpolished inner surface. They were all equipped with modified Swagelok or Parker-Hannifin compression fittings. Very thin stainless-steel mesh discs were placed at both ends of the column (part No. 206, hetp, Macclesfield, Great Britain). The valve injector was connected to the column via 1/16 in. O.D. (0.15 mm I.D.) stainless-steel tubing and the detector via 1/16 in. O.D. (0.15 mm I.D.) PTFE tubing (see Fig. 2).
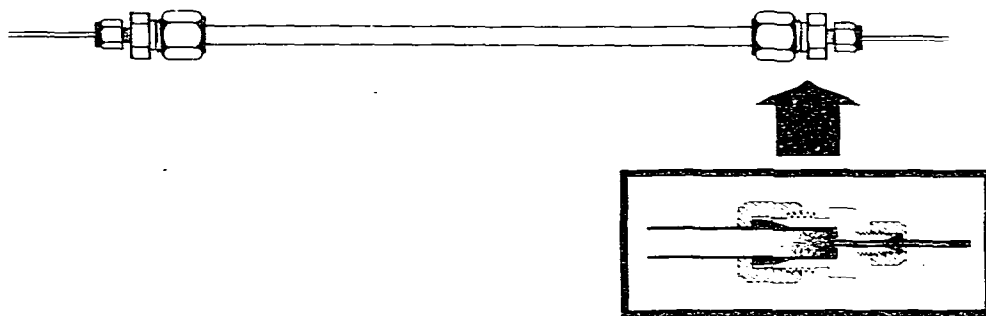

Fig. 2. Column and design of column head and bottom for valve injection.

Column packing technique

Columns were packed according to the upward slurry packing technique [13]. For reversed-phase packing materials we have found that both acetone and chloroform are suitable.

A Haskel pneumatic amplifier pump Model DST-150 (Haskel, Burbank, Calif., U.S.A.) was pressurized with solvent to 150 or 400 atm with a Whitey valve Model NB-SS-3NBF4 (Whitey, Oakland, Calif., U.S.A.), closed on the outlet side. In certain cases of packing 10 μm material at 150 atm, peak tailing was obtained. However, this could be eliminated by packing at 400 atm instead. For 5-mm I.D. columns about 2.9 g of packing material was slurried in 70 ml of solvent in an ultrasonic bath for 5 min. The slurry reservoir, Crawford Type -304-HDF4-75 (part of a slurry packing kit, part No. 316; hetp) was filled and the column mounted pointing upwards. Solvent was filled to the top of the column. The end fitting was connected and the valve was opened. About 250 ml of solvent were passed, and the column was turned pointing downwards. The valve was closed, and after 5 min the column was disassembled, washed with methanol and tested. This technique is applicable for packing both 5 and 10 μm ODS material, as well as other reversed-phase materials like LiChrosorb RP-2 and RP-8 [14]. The technique is also well suited for packing silica with methanol as slurrying medium.

Chemicals

Acetone, chloroform, phosphoric acid (85%), sodium metabisulphite, perchloric acid (75%), and sodium hydroxide were all of analytical grade (Merck).

Sodium *n*-octyl sulphate (for tenside tests, Merck) methanesulphonic acid (for synthesis, Merck), methanol (analytical reagent grade, May & Baker, Dagenham, Great Britain). 2-S-, 5-S- and 6-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa were synthesized as described by Agrup et al. [15]. DL-α-3,4-Dihydroxyphenylalanine (DL-dopa), DL-1-(3,4-dihydroxyphenyl)-2-aminoethanol hydrochloride (noradrenaline), 3,4-dihydroxyphenyl-2-methylaminoethanol (adrenaline) were all obtained from Sigma, St. Louis, Mo., U.S.A. Aluminium oxide (Merck, nach Brockmann) was prepared by the method of Anton and Sayre [16].

All the above standards were stored as such or in solution in a refrigerator. 2-S-, 5-S-, 6-S-cysteinyldopa, and 2,5-S,S-dicysteinyldopa were obtained in 0.1 *M* HCl solution and stored as such. Dopa and noradrenaline were dissolved and stored in the mobile phase. Before injections were made all standards were dissolved in the mobile phase.

Chromatographic conditions

All chromatographic experiments were performed at ambient temperature. The flow-rate was 100 ml/h unless otherwise stated. No extra precautions were necessary in order to isolate the electrochemical detector electrically.

Procedures

Before any measurements were made, a standard solution containing dopa and cysteinyldopas was injected repeatedly to establish steady-state conditions. Capacity factors* were calculated as the mean from at least two injections. The column void volume was estimated by the injection of sodium nitrate when using the UV-detector and taken as the first base-line disturbance when using the electrochemical detector.

Effect of ionic strength on retention. This was investigated for four commercially available 10-μm reversed-phase packing materials by adding sodium sulphate to an aqueous mobile phase containing 2.9 g of phosphoric acid per litre (see also Fig. 3).

Effect of pH on retention. This was studied for 5 μm Nucleosil C_{18} by adjusting the pH of the aqueous mobile phase containing either sodium sulphate or the modifier methanesulphonic acid. In the first case, the mobile phase contained 14 g of sodium sulphate per litre, and the pH was adjusted by adding phosphoric acid and 5 *M* sodium hydroxide to this solution. In the second case, the mobile phase contained 0.48 g of methanesulphonic acid and 2.9 g of phosphoric acid per litre, and the pH was adjusted with 5 *M* sodium hydroxide (see Figs. 4 and 5).

Effect of the amount of modifier on retention. This was examined for 5 μm Nucleosil C_{18} by varying the methanesulphonic acid content of the aqueous mobile phase containing 2.9 g of phosphoric acid per litre. The pH was adjusted to 1.75 with 5 *M* sodium hydroxide (see Fig. 6).

Recovery from alumina adsorption. This was investigated by treating standards according to the procedure for serum and urine [2].

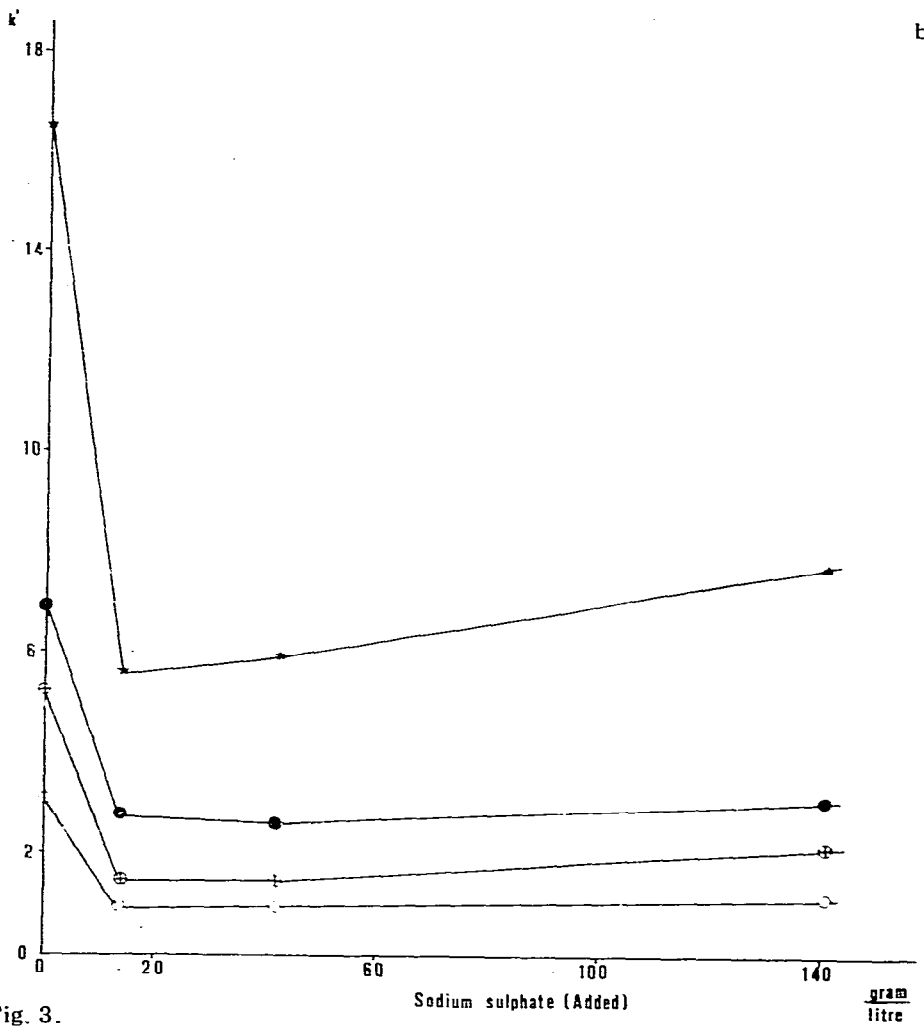*Capacity factor $k' = \dfrac{V_R - V_0}{V_0}$; V_R = retention volume; V_0 = void volume.
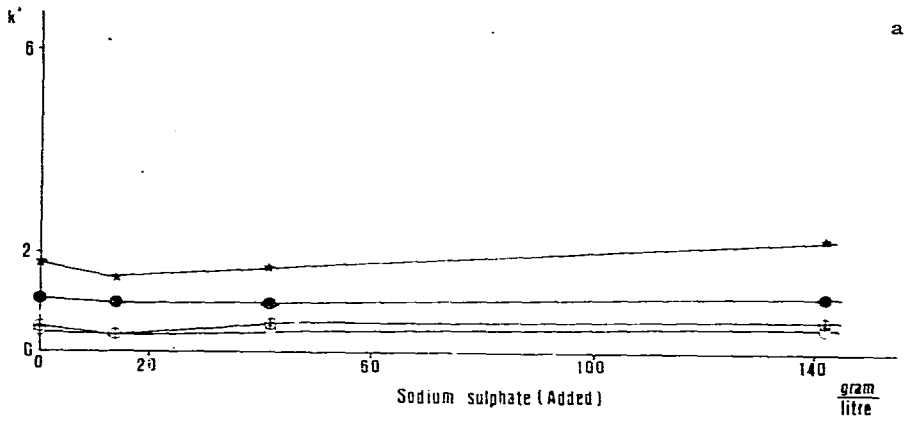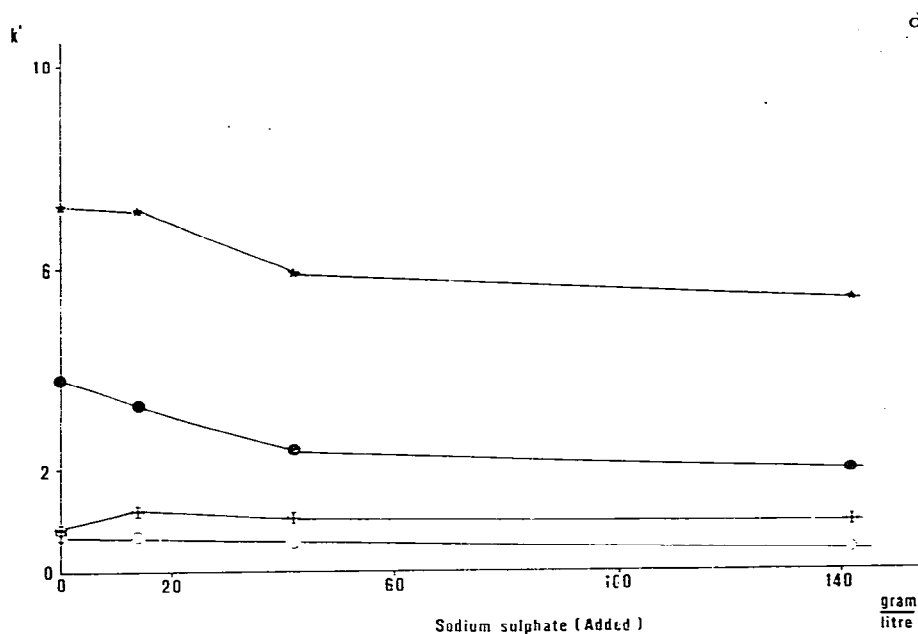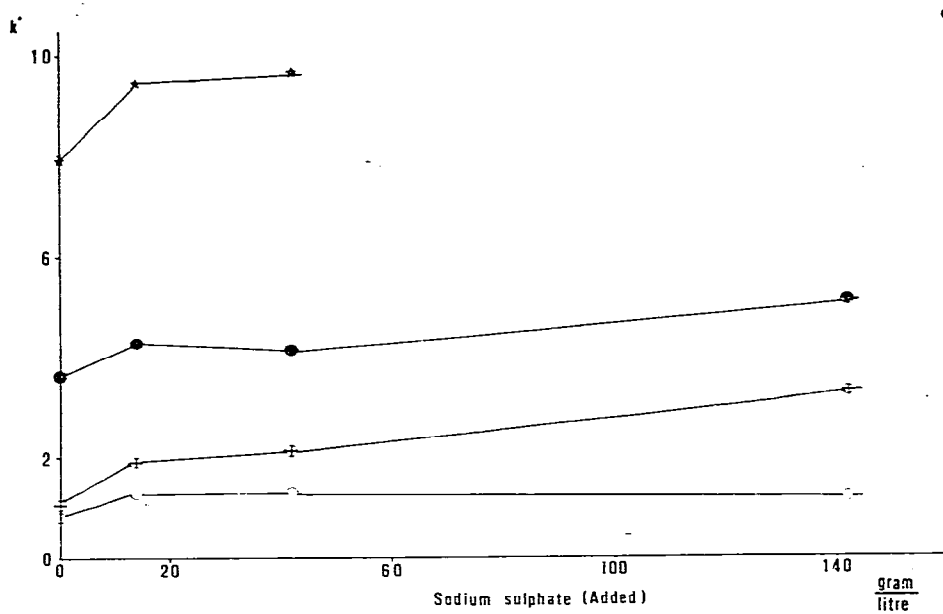
Fig. 3.

Fig. 3. Relationship between capacity factor k', and amount of sodium sulphate added to the mobile phase. Column: reversed-phase ODS materials (10 μm), 200 × 5 mm. Eluent: water, 2.9 g phosphoric acid per litre, with the addition of sodium sulphate. Flow-rate: 100 ml/h. ★, 5-S-Cysteinyldopa; ●, dopa; ·, 2-S-cysteinyldopa; +, 2,5-S,S-dicysteinyldopa. (a) Partisil ODS; (b) Spherisorb ODS; (c) Nucleosil C_{18}; (d) LiChrosorb RP-18.
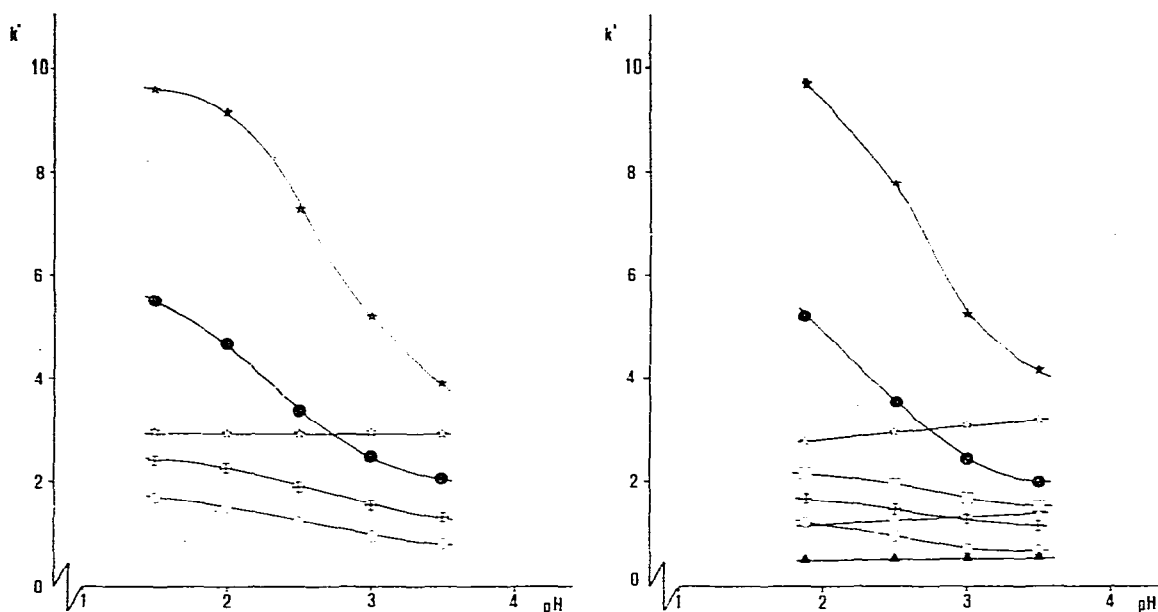
Fig. 4. Relationship between capacity factor k', and pH for cysteinyldopas, dopa and dopamine. Column: Nucleosil C_{18} (5 μm), 200 × 5 mm. Eluent: water, 14 g sodium sulphate per litre, pH was adjusted by the addition of phosphoric acid and 5 M sodium hydroxide. Flow-rate: 100 ml/h. Pressure: 168 atm. ∗, 5-S-Cysteinyldopa; ●, dopa; ○, dopamine; ∻, 2,5-S,S-dicysteinyldopa; ○, 2-S-cysteinyldopa.

Fig. 5. Relationship between capacity factor k', and pH of cysteinyldopas, dopa, dopamine, adrenaline and noradrenaline. Column: Nucleosil C_{18} (5 μm), 200 × 5 mm. Eluent: water, 0.48 g methanesulphonic acid and 2.9 g phosphoric acid per litre. pH was adjusted with 5 M sodium hydroxide. Flow-rate: 100 ml/h. Pressure: 168 atm. ∗, 5-S-Cysteinyldopa; ●, dopa; ○, dopamine; ○, 6-S-cysteinyldopa; ∻, 2,5-S,S-dicysteinyldopa; ○, 2-S-cysteinyldopa; △, adrenaline; ▲, noradrenaline.

Separation of 2-S, 5-S, 6-S-cysteinyldopa, and 2,5-S,S-dicysteinyldopa, dopa, dopamine, noradrenaline, and adrenaline. Serum and urine samples were treated as described elsewhere [2] with the exception that 1 M perchloric acid was used for elution from alumina instead of 0.1 M in order to improve recovery, especially for 2,5-S,S-dicysteinyldopa. The separation was performed on 5 μm Nucleosil C_{18} using an aqueous mobile phase with 2.9 g of phosphoric acid and 6 g of methanesulphonic acid per litre. The pH was adjusted to 1.75 with 5 M sodium hydroxide (see Fig. 7).

Characterization of reversed-phase packing materials. The carbon content of the packing material was determined in duplicate on a Model 1102 Carlo Erba elemental analyzer. For estimation of silanol interaction the columns were eluted with hexane and the capacity factors for nitrobenzene and benzene were determined [17].
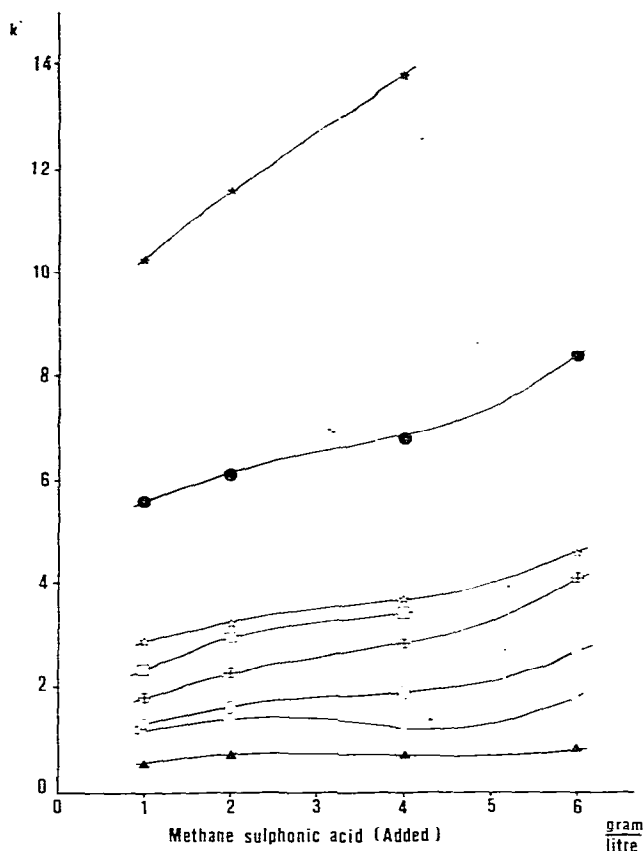
Fig. 6. Relationship between capacity factor, k', and added amounts of methanesulphonic acid to the mobile phase for cysteinyldopas, dopa, dopamine, adrenaline and noradrenaline. Column: Nucleosil C_{18} (5 μm), 200 × 5 mm. Eluent: water, 2.9 g phosphoric acid per litre. pH was adjusted to 1.75 with 5 M sodium hydroxide. Flow-rate: 100 ml/h. Pressure: 168 atm. ★, 5-S-cysteinyldopa; ●, dopa; ⋆, dopamine; □, 6-S-cysteinyldopa; ✦, 2,5-S,S-dicysteinyldopa; ⋄, 2-S-cysteinyldopa; △, adrenaline; ▲, noradrenaline.

RESULTS AND DISCUSSION

Comparison of reversed-phase packing materials and effect of ionic strength on retention

The effect of ionic strength on the retention of 2-S- and 5-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa and dopa was studied for four 10-μm commercial reversed-phase packing materials by adding sodium sulphate to the acidified aqueous mobile phase. The results are presented in Fig. 3 a—d. The names and some properties of the packing materials are given in Table I.

As can be seen from Fig. 3 a—d there are considerable differences in the k' values of the test substances on the four packing materials. As they all have octadecyl groups chemically bonded to silica this differences must be connected with the properties of the silica matrix and with the method of fixing the
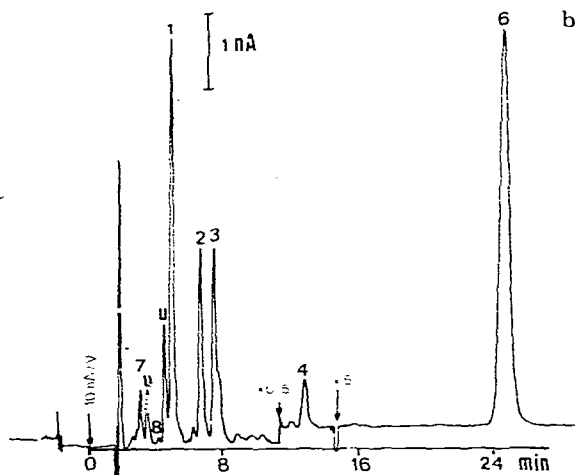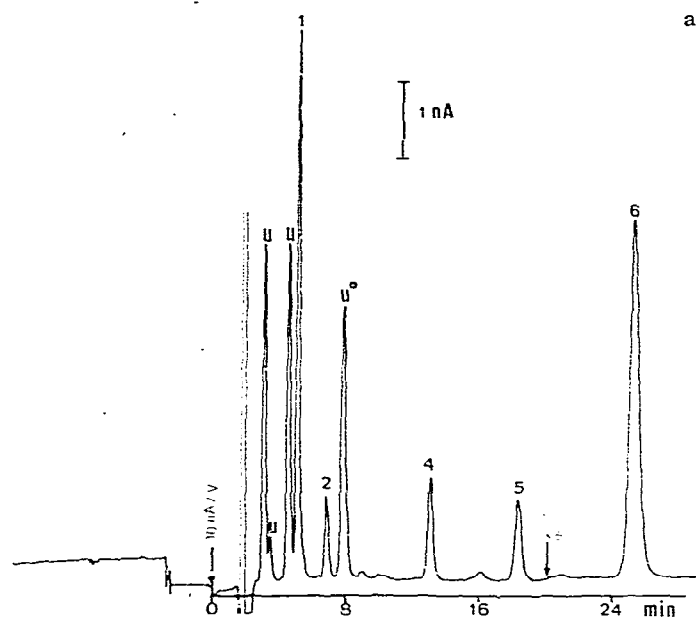
Fig. 7. Chromatograms of purified serum (a) and urine (b) obtained from patients with melanoma metastasis. Eluent: water, 2.9 g phosphoric acid and 6 g methanesulphonic acid per litre. pH was adjusted to 1.75 with 5 M sodium hydroxide. Column: Nucleosil C_{18} (5 μm), 200 × 5 mm. Flow-rate: 102 ml/h. Peaks: 1 = 2-S-cysteinyldopa; 2 = 2,5-S,S-dicysteinyldopa; 3 = dopamine; 4 = dopa; 5 = isoprenaline (internal standard); 6 = 5-S-cysteinyldopa; 7 = noradrenaline; 8 = adrenaline; u = unidentified. 6-S-cysteinyldopa is not detected, retention time between peaks 2 and 3. In contrast to peak 3 in Fig. 7b, u* does not correspond to dopamine which was shown by chromatography with other mobile phases.

TABLE I

CARBON CONTENT, SURFACE AREA AND RETENTION CHARACTERISTICS FOR DIFFERENT CHEMICALLY BONDED REVERSED-PHASE MATERIALS

Column packing material (10 μm)	Carbon content (%)	Surface area* (m² /g)	k' with hexane as eluent	
			Benzene	Nitrobenzene
Partisil ODS	4.06	300	0.15	0.80
Spherisorb ODS	6.67	220	0.14	0.58
Nucleosil C_{18}	18.6	300	0.20	0.45
LiChrosorb RP-18	19.8	400	<0.1	0.21

* According to the manufacturer.

octadecyl groups to the matrix. This latter method determines the structure of the hydrocarbon part of the packing material, which is either monomeric or polymeric [18].

It is argued that under otherwise fixed conditions the intrinsic retentive capability is higher for packing materials with higher carbon content [19]. This is not verified by the results obtained here with salt-free systems, since the highest k' values are observed for the low-carbon phase Spherisorb ODS. The considerable difference between Partisil ODS and Spherisorb ODS is also noteworthy. Although their carbon contents are of the same magnitude the latter phase gives considerably higher k' values. However, Partisil ODS, with the lowest carbon content of the phases tested, was also least retentive.

Unreacted silanol groups on the silica surface can play a role, but their effect on retention is not clear, Karch et al. [17] argued that the values of the capacity factors for benzene and nitrobenzene using nonpolar mobile phases like hexane reflect the degree of interaction with unreacted silanol groups. However, there is no correlation between the k' values in Table I and the retentions observed for the test substances on the four packing materials.

On increasing the ionic strength by addition of sodium sulphate a decrease or increase in retention results. A great decrease takes place for Spherisorb ODS while for other packing materials the change is only moderate. In general the effect on retention for the four test substances is similar on the same phase. The change in retention observed for Spherisorb ODS and to a much smaller extent for Partisil ODS is in accordance with the theory of Horváth et al. [20]. However, for Nucleosil C_{18}, retention increases with an increase in ionic strength, which is contrary to the theory. On LiChrosorb RP-18 an increase of the k' values took place for some compounds and a decrease for others. The retention differences observed for the four packing materials indicate that the nature of the reversed-phase packing material plays an important role in the retention mechanism. Horváth and Melander [19] have pointed out some properties of the packing material which can be responsible for the observed differences.

In the absence of sodium sulphate, complete separation of the test mixture was obtained for Spherisorb ODS only; for the other packing materials separation was achieved upon addition of sodium sulphate. However, the necessary amount of salt differs. Thus, on Partisil ODS 5—10 times more salt had to be added in order to accomplish separation, cf. Fig. 3 a—d. It should be noted that the test mixture contains only four of the eight compounds present in the final mixture. Of the missing compounds, adrenaline and noradrenaline do not separate but travel with the front.

The total plate number of the columns was poor in the above systems, 500—2000 theoretical plates being obtained for the test compounds at 100 ml/h, using a valve injector with a 10-μl loop. The efficiency was least for Spherisorb ODS, the number of theoretical plates only amounting to 500—800. Although the chemical nature of the packing materials is unknown, the very low efficiency for Spherisorb ODS might indicate a polymeric type of chemically bonded phase. It also has the lowest surface area among the packing materials investigated. The average low efficiency obtained could also partly be due to poor wettability of the packing material with concomitant increase in resistance to mass transfer between the phases [5].

Effect of pH on retention

The effect of pH on retention was studied either with a mobile phase having approximately constant ionic strength or with a mobile phase containing methanesulphonic acid as modifier. In both cases the packing material was 5 μm Nucleosil C_{18}. The results are presented in Figs. 4 and 5. In both cases the test mixture was complete, containing all eight compounds to be analyzed in serum and urine, except for 6-S-cysteinyldopa in the first case. Adrenaline and noradrenaline are not included in Fig. 4, as they did not separate and moved with the front in the pH range investigated.

As shown by the figures, the influence of pH on retention is considerable for dopa and 5-S-cysteinyldopa, but only moderate for the remaining amino acids in the actual pH-range. The decrease in retention is due to zwitterion formation as the isoelectric point is approached, the solubility of an amino acid going through a minimum at this point. There is no obvious explanation for the difference in pH-sensitivity between dopa and 5-S-cysteinyldopa on the one hand and the remaining amino acids on the other. However, it appears that the pH-sensitivity of dopa is greatly reduced by introducing cysteinyl groups at the 2- or 6-position. For the catecholamines dopamine, adrenaline and noradrenaline, there is a slight increase in k' values with increasing pH, reflecting the gradual transformation of substituted ammonium cations into neutral compounds. At higher pH values than those studied here, this transformation causes a steep rise in the curve due to decreasing solubility, as shown by Molnár and Horváth [7].

For these systems the efficiency was better, amounting 6000—7000 theoretical plates for the systems in Fig. 4 and about 8000 theoretical plates for those in Fig. 5. This can partly be ascribed to the fact that 5 μm particles were used. For the systems containing methanesulphonic acid, improved mass transfer between the phases also contributes to increasing the number of theoretical plates obtained.

Effect of addition of modifiers on retention and separation of cysteinyldopas, dopa, dopamine, adrenaline and noradrenaline in serum and urine ·

Sufficient resolution could not be attained for the separation of all eight compounds involved by merely varying ionic strength and pH. That an enhancement of retention of adrenaline and noradrenaline could be achieved by adding an organic modifier like methanesulphonic acid was already shown in Fig. 5. On that basis we wished to study in some detail the effect of adding anionic modifiers to the mobile phase, using 5 μm Nucleosil C_{18} as packing material.

Original attempts to use sodium *n*-octyl sulphate ($\sim$ 10 mg/l) as modifier were not quite successful. Although good separations of all compounds were obtained, steady-state conditions were achieved very slowly, indicating a successive adsorption of detergent on the surface of the packing material [6]. In addition, the peak for 2,5-S,S-dicysteinyldopa was, for unknown reasons, seriously broadened.

By using methanesulphonic acid instead, steady-state conditions were achieved much faster. Fig. 6 presents relations between k' values and concentration of methanesulphonic acid in the mobile phase for all compounds of interest. As can be seen, for most of the compounds there is an increase in retention with an increase in concentration of modifier.

On the basis of the previous experiments, conditions were chosen which permitted the separation of all catecholic amino acids studied, as well as dopamine, noradrenaline and adrenaline on 5 μm Nucleosil C_{18}. Fig. 7 a and b shows chromatograms obtained for serum and urine, respectively, from patients with malignant melanoma. All compounds except 6-S-cysteinyldopa, which was estimated to be present in too low concentration to be detected, have been identified on the basis of retention data of standards. The concentrations of dopamine, noradrenaline and adrenaline in serum are estimated to be low. Furthermore, unidentified compounds elute together with dopamine and noradrenaline. Consequently, these compounds are not visible in Fig. 7a. However, their detection in urine poses no problem [21], although a somewhat more concentrated urine sample than that represented in Fig. 7b is desirable. Additional work on the quantitative analysis of the above compound is in progress. Further improvement in resolution is necessary in order to achieve quantitative determination of all compounds involved in one run.

Recovery after adsorption on alumina

The recovery of four catecholic amino acids after adsorption on alumina was studied using three acidic eluents, and the results are given in Table II. Although 0.1 M perchloric acid was used in our previous determination of dopa and 5-S-cysteinyldopa [2], 1 M perchloric acid was chosen for the analysis of serum and urine samples, because of the higher average recovery. Use of alumina might lead to the appearance of fines in the eluate and thus filtration might be necessary. Otherwise the column can be blocked.

Electrochemical detection

The electrochemical detection of catechols is advantageous due to their low oxidation potential. We have found that the LC-10 detector ·is compatible

TABLE II

RECOVERY OF CATECHOLIC AMINO ACIDS AFTER ADSORPTION ONTO ALUMINA AND ELUTION WITH ACID

Compound	Amount added (ng)	Recovery (%)		
		0.1 M HClO$_4$	1 M HClO$_4$	35% HBF$_4$
Dopa	330	71	76	71
5-S-Cysteinyldopa	927	31	56	62
2-S-Cysteinyldopa	480	38	62	72
2,5-S,S-Dicysteinyldopa	330	11	55	47

TABLE III

EFFECT OF CHANGING WORKING POTENTIAL ON SENSITIVITY

Mobile phase: 3% (v/v) methanol in water, 2.9 g H$_3$PO$_4$ per litre. Injected amount: 5—10 ng each.

Compound	Percentage increase in peak height when changing the working potential from 0.72 V to 0.90 V
5-S-Cysteinyldopa	25
Dopa	38
Dopamine	49
2,5-S,S-Dicysteinyldopa	36

with all the chromatographic systems studied here. The combination of this detector with highly efficient columns packed with microparticulate reversed-phase material has made it possible to detect and accurately determine 25 pg of 5-S-cysteinyldopa [2].

The sensitivity of the detector can be increased by increasing the working potential. This is shown in Table III. Although sensitivity is markedly increased for the compounds investigated, selectivity will decrease with higher oxidizing potential, non-catecholic substances will also be oxidized. The sensitivity was found to differ slightly even on packing the electrode with graphite paste from the same lot. Sensitivity was also found to be higher for the first two or three injections. It thus seems advisable to make several injections on a fresh electrode in order to avoid differences in sensitivity before quantitative analysis is carried out. Due to these differences in sensitivity, an internal standard is advantageous.

CONCLUSIONS

High-performance liquid chromatography (HPLC) with a chemically bonded reversed-phase packing material combined with electrochemical detection has

been used to separate and detect the catecholic amino acids dopa, 2-S- and 5-S-cysteinyldopa and 2,5-S,S-dicysteinyldopa in serum from patients with malignant melanoma. In urine the catecholamines dopamine, noradrenaline and adrenaline were also separated, together with the above-mentioned compounds, in a single chromatographic run. Separation is achieved with the addition of methanesulphonic acid to an acidified aqueous mobile phase. By changing the pH of the mobile phase and the concentration of methanesulphonic acid, the retention can be varied, thus allowing for the optimization of the separation conditions. Modifiers with a longer lipophilic group could also be valuable in cases where a further enhancement of retention is desired, as shown by Horváth et al. [22].

A comparison of the performance of four commercial reversed-phase packing materials containing chemically bonded octadecyl groups using a standard mixture of catecholic amino acids shows that there is a considerable difference between packing materials from different manufacturers. This was also evident from a study of the variation of retention of the test mixture when the ionic strength of the mobile phase was changed by the addition of sodium sulphate.

Efficiency is markedly increased using 5 μm packing material. The addition of an organic modifier also improves efficiency, presumably due to an enhancement of mass transfer between the phases.

Systems involving combination of reversed-phase microparticulate packing materials with acidified aqueous mobile phases containing salts or organic modifiers are well suited to the analysis of catecholic compounds with electrochemical detection. It is possible to detect very small amounts of catecholic amino acids and amines, e.g. 25 pg of 5-S-cysteinyldopa. Essential for this result is the selective adsorption of catecholic compounds on alumina prior to separation by HPLC. By this method the overall sensitivity and selectivity is considerably improved.

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