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Separation of oxypurines by ligand-exchange chromatography and determination of caffeine in beverages and pharmaceuticals*

Ligand-exchange chromatography on a copper-loaded chelating resin has previously been used for the isolation of amino acids from sea water[1], the separation of peptides from amino acids[2,3], and the separation of nucleic acid components[4]. In the latter case several oxypurines were successfully separated from one another. This group of compounds is of interest because they occur naturally in the human body (disorders in purine metabolism are important medical problems) and because many of them are physiologically active and are components of pharmaceutical preparations. Caffeine, in particular, is a natural constituent of coffee beans, cola nuts, and tea, and it is an active ingredient of many commercial decongestant and analgesic tablets, and is combined with barbiturates and antibiotics in various pharmaceutical preparations.

In this note we investigated the separation by ligand-exchange chromatography of oxypurines from one another and from the other compounds with which they are associated naturally and in commercial preparations.

Experimental

The chelating resin (Chelex 100, 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.) was loaded with copper(II) ion, washed and treated with aqueous ammonia. Eluant was pumped through the packed column (packed as described previously[4]) at a constant flow rate with a Beckman Accu-Flo pump. The UV absorption of the column effluent was monitored at 260 nm with a Beckman DB-G spectrophotometer equipped with a flow cell. Data were recorded linearly in absorbance. Samples were introduced onto the column by means of an injection valve (Chromatronix Inc., Berkeley, Calif., Catalog No. SV-8031) with calibrated sample loops.

Pharmaceutical preparations were analyzed by dissolving one tablet or capsule in water, filtering out any insoluble material, and diluting to 250 or 500 ml in a volumetric flask. A 250-μl sample was injected on the column. Beverages were analyzed by injecting 250 μl on the column without any pretreatment, or if this proved to be an overload, first diluting with two volumes of water. The retention volumes of the oxypurines were determined by injecting 50 μg of sample along with 25 μg of uridylic acid, a compound which is not retained on the column and is eluted at void volume.

Results and discussion

The retention volumes determined for various oxypurines are given in Table I. Two general trends in the elution of oxypurines are evident. Methyl substituents at position 1 result in greater retention of the oxypurine, while methyl substituents at position 7 have the opposite effect. Peak width at half-height is 3 to 6 ml depending on elution position so that compounds whose retention volumes differ by 5 ml in the

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TABLE I

RETENTION VOLUMES OF OXYPURINES[a]

Compound	*Corrected retention volume*[b] *(ml)*
Purine metabolites	
Adenine	44
Guanine	30
Hypoxanthine	65
Xanthine	34
Uric acid	0
Xanthine derivatives	
Xanthosine	0
1-Methylxanthine	36
3-Methylxanthine	23
7-Methylxanthine	2
1,3-Dimethylxanthine	26
3,7-Dimethylxanthine	4
1,3,7-Trimethylxantine	68
Guanine derivatives	
Guanosine	0
Deoxyguanosine	7
1-Methylguanosine	32
N^2-Methylguanosine	7
N^2-Dimethylguanosine	3

[a] 1 × 80 cm column eluted with 1 *M* NH_3 at 0.75 ml/min.
[b] Elution volume minus the void volume.

early part of the chromatogram, and 10 ml in the later part, are well resolved. All the purine metabolites, except guanine and xanthine, are resolved from one another, as are many of the methylated xanthines. Caffeine (1,3,7-trimethylxanthine) is strongly retained on the column, complexing apparently as an uncharged species, and is well separated from theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine), and most other compounds. Consequently caffeine is readily separated and determined in real samples.

Fig. 1 shows the chromatograms obtained with four pharmaceutical preparations using a short (1 × 20 cm) column and 3 *M* NH_3 as eluant at a relatively fast flow rate of 3 ml/min. Under these conditions compounds which are only weakly retained on the column, such as aspirin, salicylamide, tetracycline, and barbiturates, appear together at, or very close to, the void volume. Caffeine elutes as a sharp peak in under 10 min. Phenacetin, a strongly basic compound, has an elution volume even greater than caffeine and appears between 15 and 30 min. Because the caffeine peak is sharp and reasonably Gaussian in shape, we constructed a calibration curve based on peak heights and quantitatively determined caffeine in the four samples with the results shown in Table II. Although a more accurate analysis can be made by peak area integration techniques, the agreement between the nominal caffeine content and that found is quite reasonable considering that a 5 to 10% variation in tablet weight is allowed. Although we only determined caffeine, it is possible to determine all three APC components from a single chromatogram, and phenacetin in the other pharmaceuticals.

Chromatograms of several different beverages were obtained with a 1 × 50 cm column, eluting with 3 *M* NH_3 at 1.3 ml/min. These are shown in Fig. 2. Conditions were chosen to provide better resolution at the front end of the chromatogram, and better separation of caffeine. Caffeine is the last peak in the chromatogram, eluting at about 45 min. Other components are obviously present in coffee and cola but we have not identified them. Quantitative results for caffeine contents are shown in

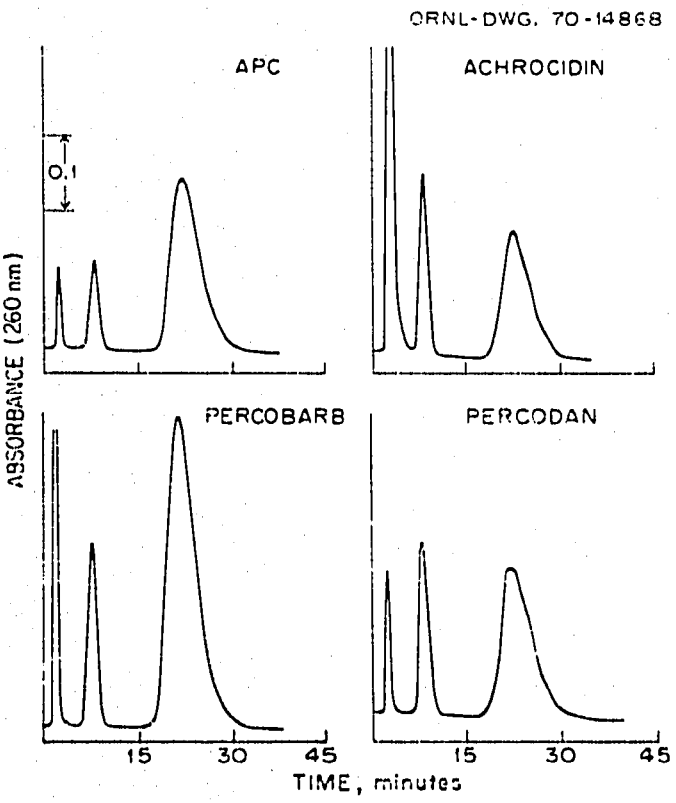

Fig. 1. Ligand-exchange chromatograms of four pharmaceutical preparations. A 1 × 20 cm column of copper(II)-loaded Chelex 100 was eluted with 3 *M* NH_3 at 3 ml/min. The sample loads of Achrocidin, Percobarb and Percodan represent 1/1000 of a tablet; that of APC represents 1/2000 of a tablet.

TABLE II

DETERMINATION OF CAFFEINE IN PHARMACEUTICAL PREPARATIONS

Preparation	*Caffeine, mg/tablet*	
	Found	*Nominal*[a]
Achrocidin	29.4	30
APC	31.6	32
Percobarb	30.7	32
Percodan	30.7	32

[a] From ref. 5.

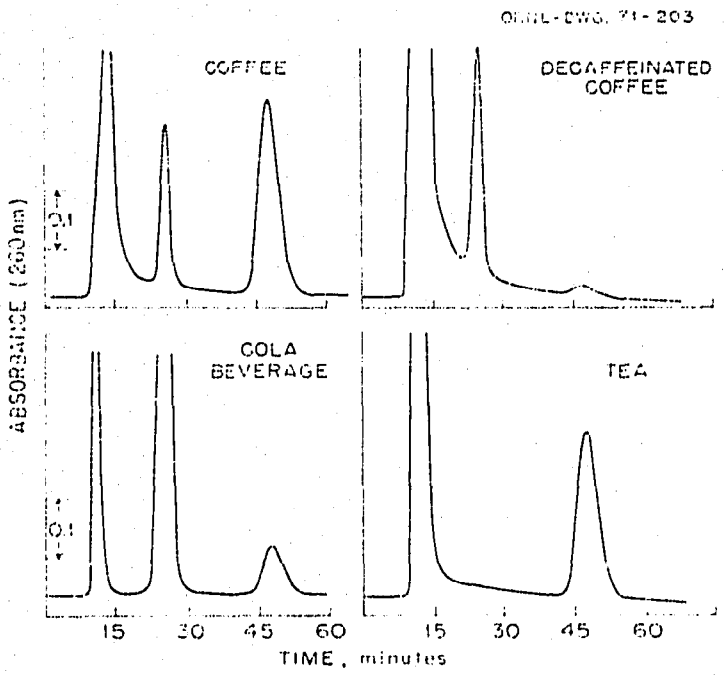

Fig. 2. Ligand-exchange chromatograms of beverages. A 1 × 50 cm column was eluted with 3 *M* NH_3 at 1.3 ml/min. Sample load was 250 μl of tea, and 125 μl of coffee and cola.

TABLE III

DETERMINATION OF CAFFEINE IN BEVERAGES

Beverage	*Caffeine content (mg per 8 oz.)*
Coffee (brewed)	160
Coffee (decaffeinated)	0
Tea (brewed)	70
Cola	40
Lemon-lime	0

Table III. The maximum caffeine content permitted in cola beverages is 0.02 % w/w (~45 mg per 8 oz.). Caffeine contents of coffee and tea are variable depending on several factors (brewed or instant, kind of tea, method of brewing, etc.). Brewed coffee usually contains about 100 mg of caffeine per cup, instant coffee about 50 mg, and tea 60–80 mg.

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