Journal of Chromatography, 166 (1978) 341–343

CHROM. 11,353

Note

Detection of inorganic sulphate and other anions on paper and thin-layer chromatograms

S. C. MITCHELL and R. H. WARING
Department of Biochemistry, University of Birmingham, Birmingham B15 2TT (Great Britain)
(Received July 25th, 1978)

Many compounds, whether endogenous, such as steroids, or exogenous, as drugs, are excreted in urine as their sulphate esters. These compounds are difficult to identify on chromatograms, since existing spray reagents are non-specific or show low sensitivity. The method described here involves elution of the sulphate conjugate and its enzymic hydrolysis. Chromatography is used to separate the aglycone from the sulphate anion, which is detected with a barium chloride–potassium permanganate spray reagent.

EXPERIMENTAL

Confirmation for compounds which are thought to be sulphate conjugates on chromatography is achieved by their elution with acetate buffer (0.075 *M*, pH 5.5). The solution is then hydrolysed with sulphatase (1000 Fishman units from *H. pomatia*, Sigma type H-1) by incubation at 37° for 18 h. Aliquots are evaporated to a small, known, volume and then run on a thin-layer chromatographic (TLC) plate in a suitable solvent (*e.g.* chloroform–methanol, 9:1). Whatman No. 1 paper with isobutyric acid–1 *M* NH_4OH (5:3) may also be used. In both cases, the aglycone has a much higher R_F value than the inorganic sulphate, which is left near the origin.

The chromatogram is dried in a current of warm air and sprayed lightly but evenly with a saturated solution of barium chloride and potassium permanganate (3.0 g $BaCl_2$ + 0.6 g $KMnO_4$ in 10 ml water, decanted before use). TLC plates are then oversprayed with HCl (9.7 *M*) and heated at 120° for 15 min. Paper chromatograms are oversprayed with HCl (4.0 *M*) and left to dry (*ca.* 3 h) at room temperature.

The sulphate ion is easily detected as a dark purple spot on a pink ground on TLC plates or as a bright pink spot on a buff ground when paper chromatograms are used (in the latter case the background is initially brown but becomes white on standing). Other common anions, including those which also contain sulphur, such as sulphite or thiosulphate, give different colour reactions (Table I) which are distinctive and stable for up to 12 months. The lower limit of detection of sulphate is 0.3 μg.

TABLE I

COLOUR REACTIONS OF SOME ANIONS WITH THE BARIUM CHLORIDE–POTASSIUM PERMANGANATE SPRAY, BEFORE AND AFTER OVERSPRAYING WITH ACID AND HEATING

— indicates indistinguishable from background.

Ion	*TLC*		*Paper*	
	Before (ground: dark purple)	*After (ground: light pink)*	*Before (ground: dark purple)*	*After (ground: white)*
F^-	purple	white/pink halo	pale pink	pale pink
Cl^-	pale mauve	dark pink	—	—
Br^-	pink/brown	pink	—	—
I^-	green	light yellow	light brown	brown
IO_3^-	—	pale pink	—	pale yellow
CO_3^{2-}	very pale pink	white	—	—
NO_3^-	pink	very pale yellow	—	—
HPO_4^{2-}	very pale lilac	—	pink	—
S^0	yellow	pale yellow	yellow	yellow
S^{2-}	bright yellow/green	white	green	light violet
SO_3^{2-}	light brown/yellow	pink	khaki	pale violet
SO_4^{2-}	dark purple	purple	pink	cyclamen pink
$S_2O_3^{2-}$	khaki	white	yellow/brown	buff
$S_2O_4^{2-}$	yellow/white	—	light green brown	—
$S_2O_5^{2-}$	khaki	pink	brown/green halo	pale pink
urea	—	—	—	—
citrate	pale yellow	pale yellow	yellow	—

DISCUSSION

Few methods are available for detecting non-radioactive sulphate in chromatography. Organic sulphates have been detected on paper using a method developed by Schneider and Lewbart[1] where hydrolysis by dioxan–HCl was followed by reaction with sodium rhodizonate[2,3]. Another method, detecting steroid sulphates, has been described where a chloroform-soluble steroid sulphate–methylene blue complex is eluted and counterstained with rhodamine[4–6]. This was modified by Nader and Dietrich[7] for inorganic sulphate, using toluidine blue O as the complexing reagent.

It was noted as early as 1829[8] that barium sulphate carried down other salts during its precipitation. Potassium permanganate was shown to co-precipitate with barium sulphate, the resulting violet colour of the precipitate being resistant to the usual reagents for permanganate reduction[9,10], and this finding was developed into spot tests for barium and sulphate by Feigl and Aufricht[11,12]. It has been shown that the potassium ion, with almost the same ionic radius as Ba^{2+} (K^+, 1.33 Å; Ba^{2+}, 1.35 Å) is required for integration into the lattice; sodium permanganate, with the same charge but a different ionic size, is ineffective[13].

The procedure described here therefore involves precipitation of barium sulphate when the barium chloride in the spray reacts with inorganic sulphate. This solid protects the co-precipitated potassium permanganate from reduction by the hydrochloric acid, so that sulphate spots are visualised as purple/pink on a pale

ground. The specificity and sensitivity of this method should prove useful for studies on sulphate metabolism and the excretion of sulphate conjugates, and provides rapid spot tests to distinguish between many common anions.

REFERENCES

1 J. J. Schneider and M. L. Lewbart, *J. Biol. Chem.*, 222 (1956) 787.
2 J. Feigl, *Mikrochemie*, 3 (1924) 187.
3 G. Gutzeit, *Helv. Chim. Acta*, 12 (1929) 736.
4 A. J. Vlitos, *Contr. Boyce Thompson Inst.*, 17 (1953) 127.
5 A. B. Roy, *Biochem. J.*, 62 (1956) 41.
6 O. Crépy and O. Judas, *Rev. Fr. Etud. Clin. Biol.*, 5 (1960) 284.
7 H. B. Nader and C. P. Dietrich, *Anal. Biochem.*, 78 (1977) 112.
8 E. Turner, *Phil. Trans. Roy. Soc. London*, 119 (1829) 291.
9 H. E. Wohlers, *Z. Anorg. Chem.*, 59 (1908) 203.
10 H. G. Grimm, C. Peters and H. Wolff, *Z. Anorg. Chem.*, 236 (1938) 57.
11 F. Feigl and W. Aufricht, *Rec. Trav. Chim.*, 58 (1939) 1127.
12 F. Feigl and W. Aufricht, *Analyst (London)*, 65 (1940) 251.
13 H. Yagoda, *J. Ind. Hyg. Toxicol.*, 26 (1944) 224.