Journal of Chromatography, 202 (1980) 494–499
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,278

Note

Routine high-performance liquid chromatographic method for assessing sorbate in tobacco*

KLAUS D. BRUNNEMANN*,** and LILIAN POSSET
Philip Morris Europe, Research Division, 2003 Neuchâtel (Switzerland)
(Received August 22nd, 1980)

Sorbic acid which occurs naturally as its lactone in the berries of the mountain ash (*Sorbus aucuparia* L.) is considered to be an effective antimycotic agent. Its potassium salt is widely used in foodstuffs[1,2] and wine[3,4] and has also been found effective in tobacco preparations and reconstituted tobacco, preventing the formation of moulds and yeasts[5]. Interestingly, sorbate is also an effective inhibitor of nitrosamine formation[6].

Our objective was to find a sensitive and specific method of determining sorbate. Two spectrophotometric methods are available. One employs ultraviolet absorption at 263 nm, taking advantage of the high molar extinction coefficient of sorbic acid[1,2], $\varepsilon = 2.6 \cdot 10^4$ l mol^{-1} cm^{-1}. This method appears to be attractive but suffers from interference from other preservatives (such as parabens) which also absorb at 263 nm. The other method makes use of a colour reaction of malonaldehyde (the oxidation product of sorbic acid) with 2-thiobarbituric acid; the coloured product is detected spectroscopically at 432 nm[1,2]. Harvey *et al.*[5] modified this colorimetric procedure for automation on the Auto Analyzer. Recently, gas chromatographic techniques for the determination of sorbate in foodstuffs have also been reported[7,8].

In order to evaluate different analytical procedures, we compared the above two optical methods with two high-performance liquid chromatographic (HPLC) methods: reversed-phase HPLC of tobacco extracts without any clean-up and reversed-phase HPLC of steam-distilled samples. We initially analyzed the tobacco extracts, to which different amounts of sorbate had been added, to ensure good homogeneity of the samples. (The extractant was water, ten times the weight of tobacco, for 1 h at 90°C; no further clean-up.) We observed that both the UV and the colorimetric methods gave results in good agreement with the HPLC method when steam-distilled samples were analyzed, whereas the direct HPLC method yielded lower values (Table I).

However, when the tobacco of some commercial cigarettes was analyzed, we also observed some interferences with both the UV and the colorimetric methods; samples in which sorbate was known to be absent did show some background using

* Presented at the *33rd Tobacco Chemists' Research Conference, Lexington, KY, October 29–31 1979.*

** Present address: Naylor Dana Institute, Valhalla, NY 10595, U.S.A.

0021-9673/80/0000–0000/$02.25

the colorimetric method (Table II). For this reason, the HPLC method described in this paper became the method of choice.

MATERIALS AND METHODS

Apparatus

For the UV analyses, we used a Beckman ACTA M IV spectrophotometer. HPLC analyses were performed on a Hewlett-Packard Model 1084 A instrument equipped with a 79875 A variable-wavelength detector and automatic sampler/injector. The columns used were a μBondapak C_{18} (Waters No. 27324) and a LiChrosorb RP-8 (Hewlett-Packard No. 79918 A); the steam generator used was a Büchi Model DG-1500.

Reagents

Potassium sorbate (No. 85520), ammonium acetate (No. 09690), phosphoric acid (No. 79620), magnesium sulphate (No. 63140), methanol (No. 65541), 2-thiobarbituric acid (No. 88481) and potassium dichromate (No. 60190) were purchased from Fluka (Buchs, Switzerland); sulphuric acid (No. 731) was obtained from E. Merck (Darmstadt, G.F.R.).

Cigarettes

The cigarettes and pipe tobaccos were purchased in different European countries during the first period of 1979.

Analytical procedure

Preparation of standard solutions. A 1.33-g amount of potassium sorbate and 10 ml 1 *N* HCl was diluted to 1 l to give a stock solution containing 1000 ppm sorbic acid. Working standards (1 and 5 ppm sorbic acid respectively) were then prepared by dilution of 0.25 ml stock solution and 3 ml 1 *N* HCl to 250 ml and of 1.25 ml stock solution and 3 ml 1 *N* HCl to 250 ml. All standard solutions may be stored for several weeks in a refrigerator in the dark (after 7 weeks, the potency of the working standards was still greater than 98%).

Preparation of samples. Fifteen grams of tobacco, 75 g $MgSO_4 \cdot 7H_2O$ and 50 ml of 0.5 *N* sulphuric acid were placed in a 500-ml flask and 494 ml water were steam distilled into a 500-ml volumetric flask containing 6 ml 1 *N* HCl. A 10-μl aliquot was then injected into the liquid chromatograph.

For the tobacco smoke preparations, the cigarettes were stored for 24 h at 60% relative humidity. Ten cigarettes were smoked under standard conditions[9] and the Cambridge filter pads used were steam distilled and worked up in the same way as the tobacco samples.

HPLC conditions. Column: LiChrosorb RP-8 or μBondapak C_{18}. Mobile phase: 75% 0.005 *M* CH_3COONH_4, adjusted to pH 4.0 with H_3PO_4 (50°C); 25% methanol (40°C). Flow-rate: 1.5 ml/min. Column pressure: 82 bar. Column temperature: 55°C.

Typical chromatograms of commercial cigarette and pipe tobaccos both with and without sorbate are shown in Fig. 1.

RESULTS AND DISCUSSION

Table I shows a comparison between the different methods employed. It is apparent that the UV and colorimetric methods and the HPLC method using steam-distilled samples give results in good agreement, whereas the direct injection of tobacco extracts yielded consistently lower values for which reason this method was abandoned. However, when batches of commercial cigarettes from various European countries were analyzed for their sorbate content we observed that steam-distilled tobacco samples gave higher values than the the UV method. Also, the colorimetric method showed elevated values when compared to the HPLC method, and samples in which sorbate was known to be absent showed some background when applying the colorimetric method (a reddish pink colour). Because of these interferences, steam distillation followed by HPLC was subsequently used. We also studied the use of an internal standard such as benzoic acid and several parabens. Although these compounds can be steam distilled, we observed that the sorbate values are affected by the presence of these compounds.

A summary of the data is shown in Table II. The contents of potassium sorbate observed in commercial cigarettes range from 48 to 81 μg per g tobacco (36 to 61 μg sorbic acid per g tobacco). Using the HPLC method, we also analyzed sixteen pipe tobaccos from various European countries; only four of them contained potassium sorbate. The levels were 8.2, 48, 95 and 221 μg per g tobacco (see also Fig. 1). The sensitivity of the assay allows the detection of 1 μg sorbate per gram of tobacco.

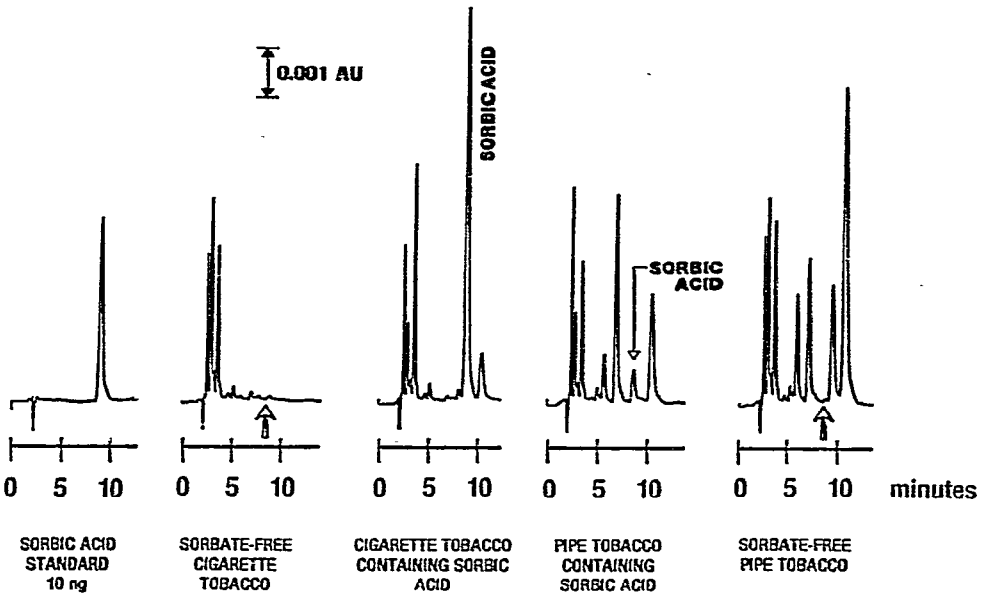

Fig. 1. Typical HPLC traces. Large arrows indicate retention time of sorbic acid.

TABLE I

COMPARISON OF DIFFERENT METHODS OF ANALYSIS OF POTASSIUM SORBATE (%) ADDED TO TOBACCO EXTRACTS

The extractant was water, ten times the weight of tobacco, for 1 h at 90°C without any further clean-up; different amounts of sorbate were then added to the extract.

Sample	*Steam distillation*			*Direct HPLC*
	UV method	*Colorimetric method*	*HPLC*	
1	0.94	0.96	0.95	0.89
2	0.040	0.040	0.038	0.031
3	0.32	0.32	0.32	0.25
4	0.54	0.51	0.51	0.44
5	0.16	0.16	0.15	0.07

TABLE II

COMPARISON OF ANALYSIS OF POTASSIUM SORBATE IN COMMERCIAL CIGARETTE TOBACCO (μg/g)

F = Filter; NF = non-filter; M = menthol; n.d. = not detected.

*Brand**			*Steam distillation*		
			UV method	*Colorimetric method*	*HPLC*
a,	80,	F, M	67.4	76.1	55.9
b,	80,	F	72.5	73.7	51.6
c,	70,	F	**	8.0	n.d.
d,	70,	F	**	7.8	n.d.
e,	70,	NF	**	9.2	n.d.
f,	90,	F	**	6.2	n.d.
g,	100,	F	**	9.0	n.d.
h,	100,	F, M	**	14.1	n.d.
i,	100,	F	56.7	58.3	48.0
j,	85,	F	**	16.8	n.d.
k,	85,	F	**	10.0	n.d.
l,	85,	F	53.1	66.9	52.7
m,	80,	F	**	19.4	n.d.
n,	80,	F	**	14.6	n.d.
o,	85,	F	**	12.9	n.d.
p,	85,	F	**	14.3	n.d.
q,	85,	F	**	17.4	n.d.
r,	100,	F	**	14.6	n.d.
s,	85,	F	**	10.9	n.d.
t,	85,	F	91.4	69.1	49.2
u,	85,	F	**	17.4	n.d.
v,	85,	F	91.9	89.6	80.8

* Number indicates length in mm.

** Interference.

In order to study the precision of the HPLC assay, we analyzed ten consecutive samples comprising 5 mg sorbic acid added to 15 g sorbate-free tobacco and obtained a coefficient of variation of 1.54% with an average recovery of 92.8% (Table III). At other sorbic acid concentrations, ranging from 0.5 to 10 mg, the recovery was

TABLE III

PRECISION OF SORBATE ASSAY

A 5.000-mg amount of sorbic acid was added to 15 g tobacco and worked up as described under *Analytical procedure*.

Sample	*Sorbic acid found (mg)*	*Recovery (%)*
1	4.702	94.0
2	4.699	94.0
3	4.661	93.2
4	4.722	94.4
5	4.695	93.9
6	4.503	90.1
7	4.566	91.3
8	4.660	93.2
9	4.628	92.6
10	4.580	91.6
Mean	= 4.652	Mean = 92.8
σ	= 0.0714	
Rel. S.D.	= 1.54%	

between 92.8 and 96.3%, with an overall average of 94.0% (Table IV). Since the recovery of sorbate is very consistent, we decided to use a factor of 94% recovery in our calculations. A lower factor must be used when less than 75 g of $MgSO_4 \cdot 7H_2O$ are used in the steam distillation step.

TABLE IV

RECOVERY OF SORBIC ACID

From 15 g of sorbate-free tobacco.

Sample	*Sorbic acid (mg)*		*Recovery (%)*
	Added	*Recovered*	
1	0.500	0.466	93.1
2	1.000	0.949	94.9
3	2.000	1.927	96.3
4	5.000	4.642	92.8
5	10.000	9.310	93.1
		Mean = 94.0	

Finally, we studied the possible transfer of sorbate from tobacco into mainstream smoke. For this experiment we impregnated tobacco with a potassium sorbate solution (40 μg sorbate per g tobacco). We observed a transfer of 12.9–14.4% sorbate into the smoke when using cigarettes with cellulose acetate filter-tips and 21.4% sorbate for plain cigarettes. This degree of transfer is similar to that of nicotine.

ACKNOWLEDGEMENT

The authors wish to thank Mr. M. Hofer for his technical support.

REFERENCES

1 F. S. Nury and H. R. Bolin, *J. Food Sci.*, 27 (1962) 370.
2 H. Schmidt, *Z. Anal. Chem.*, 178 (1960) 173.
3 F. Eisenbeiss, M. Weber and S. Ehlerding, *Chromatographia*, 10 (1977) 262.
4 G. Ziemelis and T. C. Somers, *Amer. J. Enol. Vitic.*, 29 (1978) 217.
5 W. R. Harvey, P. G. Baker and W. B. Fountaine, *Tob. Sci.*, 19 (1975) 133.
6 K. Tanaka, K. C. Chung, H. Hayatsu and T. Kada, *Food Cosmet. Toxicol.*, 16 (1978) 209.
7 L. C. Zygmunt, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 939.
8 W. Rauter and W. Wolkerstorfer, *Z. Lebensm.-Unters.-Forsch.*, 169 (1979) 435.
9 K. Brunnemann, D. Hoffmann, E. L. Wynder and G. B. Gori, in E. L. Wynder, D. Hoffmann and G. B. Gori (Editors), *Proc. 3rd World Conf. on Smoking and Health*, U.S. Govt. Printing Office, Washington, DC, 1976, pp. 441–449.