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DETERMINATION OF CYCLAMATE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH INDIRECT PHOTOMETRY

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SUMMARY

Most artificial sweeteners have been determined by high-performance liquid chromatography, but not cyclamate. We propose a simple method using standard equipment without any chemical reaction involving the cyclamate ion by applying the technique of indirect photometry. Saccharin, dulcin and aspartam may also be determined, if present.

INTRODUCTION

Artificial sweeteners play an important role in nutrition not only for diabetics but also for people who are looking more and more for low-calorie foods. The commonest sweetners are saccharin and cyclamate; others are dulcin, aspartame and acesulfam (acetosulfam). The quantitative analysis of most of these substances is possible today using the reliable and rapid technique of high-performance liquid chromatography (HPLC)[1–6]. But such a method has not yet been described for cyclamate, essentially because it does not show absorption in the essentially used UV range (above 200 nm). Apart from the tedious analysis of denaturated cyclamic acid (sulfate precipitation, coloured reaction on thin-layer plates, etc.) only a gas chromatographic determination of the corresponding amine has been published[7]: extraction and chemical reactions are, however, necessary. The lack of absorption is a problem encountered with alot of substances, and to overcome the difficulty of their detection with liquid chromatography, indirect photometry has been proposed by Small[8] in conjunction with conventional ion exchange chromatography, and by Deckert (ref. 9 and references therein) and Dreux[10] with high-performance ion-pair liquid chromatography systems.

The principle is simple: the eluent containing ions of suitable absorptivity produces a strong absorption background; if ions with smaller absorptivity are eluted, they cause a negative deflection of this signal at the photometer. This technique is used more and more for the analysis of inorganic ions and we propose an application for the determination of cyclamate in drinks and other dietetic foods. Under the same conditions saccharin and dulcin can also be determined, as a positive signal owing to their absorptivity at the working wavelength. Changing the conditions a

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little enables the analysis of aspartame together with the other sweeteners in a run of *ca.* 24 min.

INDIRECT PHOTOMETRY OF PAIRED IONS

Ion system

An ion-paired separation of the cyclamate anion needs a suitable pairing cation. We chose the tetrabutylammonium (TBA) cation, not only because it is the most used pairing ion but also because it attains adsorption equilibrium very rapidly compared with other species, such as the cetrimide cation (N-cetyl-N,N,N-trimethylammonium)[11].

The counter-ion with high absorptivity producing the necessary background should be monovalent to obtain the best sensitivity: owing to the conservation of the electroneutrality of the solute, cyclamate ions replace an equivalent ion quantity of the counter-ion. A tentative assay with inorganic iodide was not successful owing to the instability of the absorption signal. Using *p*-toluenesulphonate we obtained very satisfactory results.

The optimal concentration of the pairing ion in the aqueous phase has been derived from the adsorption isotherms given by Hung and Taylor[12] with TBA on C_{18} modified silica and lies in the millimole range. Table I gives the theoretical plate number we obtained with the first column (self-packed) for cyclamate and saccharin with 3, 5 and 8 m*M* solutions of *p*-toluenesulphonate mixed with 12% methanol as mobile phase. In the best case the height of an equivalent plate is less than 0.1 mm for cyclamate, that is quite reasonable for an ion-exchange system. Saccharin is eluted undissociated and separated on a reversed-phase principle.

TABLE I

PLATE NUMBERS FOR VARIOUS CONCENTRATIONS OF TETRABUTYLAMMONIUM *p*-TOLUENESULPHONATE IN THE AQUEOUS PHASE

RP-18 column, 12% methanol.

Concentration (mM)	*Cyclamate*	*Saccharin*
3	1100	750
5	1500	1100
8	800	600

Indirect photometry

The absorption signal of the eluent should be compensated at the spectrophotometer, enabling measurements with the extinction range set at least at 0.05 units. The best sensitivity is obtained by measuring at the absorption maximum wavelength of *p*-toluenesulphonate (λ = 260 nm), but owing to the electronic capabilities of the spectrophotometer for compensation, the wavelength of measurement must be adapted to a higher value. The pen of the recorder is set in the middle of the paper (offset) so that positive and negative signals can be plotted. If one uses a computer for the integration of the curves, care must be taken that negative deflections are properly transmitted through the analog–digital interface.

EXPERIMENTAL

Chemicals

Tetrabutylammonium *p*-toluenesulphonate (Fluka No. 86887, F.R.G.); cyclamic acid, sodium salt (Sigma No. C9131, U.S.A.); saccharin, sodium salt (Sigma No. S1002, U.S.A.); dulcin (*p*-phenetylurea; Sigma No. D6631, U.S.A.); aspartam (L-aspartyl-L-phenylalaninemethyl ester, EGA-Chemie No. 85890-0, Steinheim, F.R.G.); glycin (Merck No. 4201, F.R.G.); methanol and water were of HPLC grade.

Samples

Liquid samples such as drinks, fruit juices or water-soluble preparations (coffee sweeteners) should be filtered if necessary and are injected directly, usually in aliquots of 30 μl.

Thick samples such as yoghurt or solid samples such as biscuits must be suspended in hot water (10–40 g), homogenized, cleared with 5 ml of both Carrez solutions (first add 2.5 ml of Carrez I solution (150 g of $K_4[Fe_4(CN)_6] \cdot 3H_2O$ in 1 l of water), stir, then add 2.5 ml of Carrez II solution (300 g of $ZnSO_4 \cdot 7\ H_2O$ in 1 l of water) and stir again), and made up to 100 ml with water. After filtration (folded filters) 30 μl are usually injected. If the sample cannot be cleared sufficiently or if the concentration of cyclamate in the sample is not high enough, then the sample should be extracted with ethylacetate[13] and the final solution concentrated prior to injection.

Apparatus and conditions

The HPLC system consists of a double-cylinder pump (Altex 100 A), an injector (Rheodyne, Model 7105) and a UV-visible spectrophotometer (Kontron, Uvikon, Switzerland). The wavelength is set at nearly 260 nm and the extinction range at 0.05. Our measurements have been made at 267 nm.

Two columns have been used. The first is cheaper than the second and is sufficient for the analysis of cyclamate and saccharin; it is 12.5 cm × 4.6 mm I.D. (Knauer, Oberursel, F.R.G.) packed in our laboratories with 10 μm LiChrosorb RP-18 (Merck No. 9334). The second column is recommended for the analysis of the four sweeteners in one run: 10 cm × 4 mm I.D. (Knauer No. 441,514,124) packed with Hypersil MOS (equivalent to RP-8) 3 μm.

The eluent is a solution of 5 m*M* tetrabutylammonium *p*-toluenesulphonate buffered with 10 m*M* glycine, set at pH 3.5 with HCl, and mixed with 12% methanol. The mixture is filtered prior to use (Millipore, 1 μm). The flow-rate is 2.0 ml/min with the first column and 1.5 ml/min with the second. About 30 min are necessary to equilibrate the columns.

For a better separation of aspartam with the MOS column it is better to elute with the salt solution mixed with 8% methanol.

After use the column should be washed first with water and then with water–methanol (70:80) before storage.

RESULTS

Fig. 1 was obtained with the commercially packed column (MOS) and shows the separation of the four sweeteners: 30 μl of a mixture containing 1000 ppm each

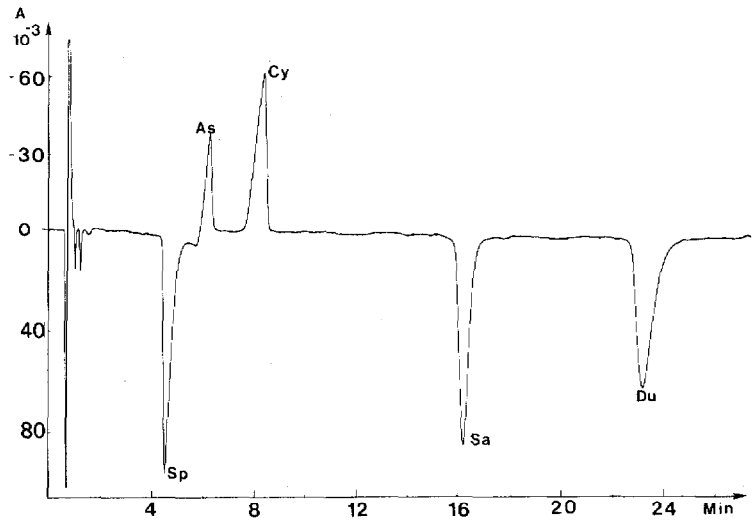

Fig. 1. Sample, 30 μl of 1000 ppm each of aspartam and cyclamate and 300 ppm each of saccharin and dulcin. Column: MOS, 3 μm, 10 cm × 4 mm I.D.; eluent 5 m*M* TBA *p*-toluenesulphonate with 8% methanol; flow-rate 1.5 ml/min.

of cyclamate and aspartam and 300 ppm each of saccharin and dulcin. The eluent was a 5 m*M* TBA *p*-toluenesulphonate water solution with 8% methanol at a flow-rate of 1.5 ml/min. The peak SP, characteristic for our analytical conditions, is called "system peak" by Denkert *et al.*[9] and may appear positive as well as negative depending on the total ion concentration of the injected sample.

Fig. 2 shows the chromatogram obtained with 30 μl of a standard solution of 1000 ppm of cyclamate and 500 ppm of saccharin with the RP-18 column.

Fig. 3, which is not drastically different from Fig. 2, was obtained by injecting 30 μl of an orange drink after filtration. This drink is declared to contain 0.5 g/l of cyclamate and 0.1 g/l of saccharin (first column).

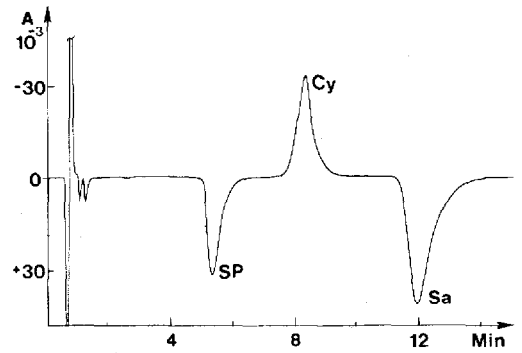

Fig. 2. Sample, 30 μl of 1000 ppm of cyclamate and 500 ppm of saccharin solution. Column: RP-18, 10 μm, 12 cm × 4.6 mm I.D.; eluent, 5 m*M* TBA *p*-toluene-sulphonate with 12% methanol; flow-rate 2.0 ml/min.

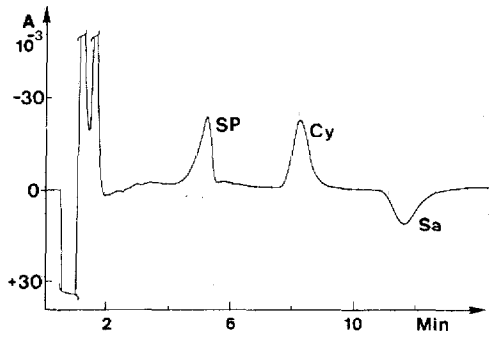

Fig. 3. Sample, 30 μl of a filtered orange drink, declared to contain 0.5 g/l of cyclamate and 0.1 g/l of saccharin. Conditions as in Fig. 2.

Fig. 4 shows the signals measured with 30 μl of dietetic yoghurt solution: 40 g of sample, cleared as described above, reduced to 100 ml and then filtered (first column).

DISCUSSION

Sensitivity

The applicability of this method is limited by its sensitivity: for a typical injection of 30 μl the final solution must contain not less than 200 ppm of cyclamate with the first column and 50 ppm of cyclamate with the second column. However, the detection limit may be enhanced if the sensitivity of the detector can be set lower than 0.05 absorption units at 260 nm. This is possible with a spectrophotometer having a large dynamic range or by applying an offset voltage at the input of the recorder to compensate for the high background signal. In the latter case the limits of the linearity of the Lambert–Beer law should be kept in mind.

For saccharin and dulcin the limit of detection is *ca.* five times better than for cyclamate, owing to their relatively strong absorption at 260 nm.

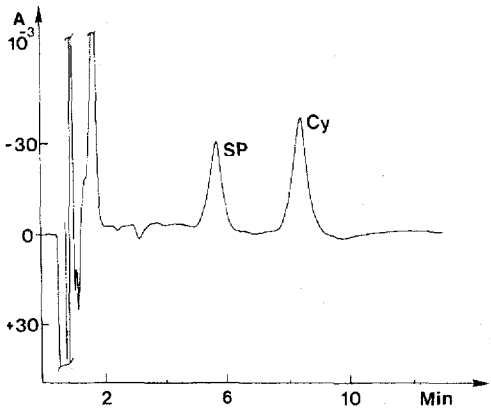

Fig. 4. Sample, 30 μl of cleared solution from 40 g of dietetic yoghurt declared to contain 2.1 g/kg of cyclamate. Conditions as in Fig. 2.

TABLE II

MEAN AND STANDARD DEVIATION OF RECOVERY FOR TYPICAL ENRICHED SAMPLES

	*Cyclamate**	*Aspartam***	*Saccharin***	*Dulcin**
Yoghurt	95 ± 2	95	87 ± 3	92 ± 2
Marmalade	93 ± 2	89	94 ± 2	90 ± 4
Chocolate	91 ± 2	95	90 ± 6	85 ± 2

* Five analyses each.
** One analysis each.

Quantitation

The extraction of the non-liquid samples has been tested with enriched yoghurts, marmalades and chocolates. The overall mean recovery obtained with 48 added samples is 91% with a standard deviation of ±4%. Table II gives the detailed values for each sweetener.

We tested the linearity of the integrated negative signals by injecting 30 μl of 200–5000 ppm cyclamate salt solution using the RP-18 column. In this concentration range the linearity is very good although the peak widths vary slightly with the amount injected. The peak height should thus not be used for quantitative determination. If it is not possible to integrate the signals properly, the other way is to prepare the sample so that the final concentration of the sweeteners (in most cases already known) approximatively falls within ±20% of the calibrated concentration and to compare the peak heights directly.

Aspartam absorbs at the working wavelength and, depending on the salt concentration of the eluent, the signal may be positive *or* negative.

Buffer

The buffering at pH 3.5 is not necessary for many foods: fruit juices, drinks (even colas) and biscuits, for example, do not need glycine in the eluent. But all the milk products (yoghurt, curds, etc.) must be analysed in acidic eluent. It may be of possible help, when working with acid-rich samples, to set the pH at a lower value, *i.e.* pH 2. This should not disturb the general form of the chromatogram for standard solutions: cyclamate remains completely dissociated and saccharin acid undissociated.

REFERENCES

1 L. Fox, G. D. Anthony and E. P. K. Lau, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 1048.
2 P. Beaud, Laboratoire Cantonal, Lausanne, personal communication.
3 M. Uihlein, *Deut. Lebensm.-Rundschau*, 73 (1977) 157.
4 H. Grosspietsch and H. Hachenberg, *Z. Lebensm.-Unters.-Forsch.*, 171 (1980) 41.
5 D. S. Smyly, B. B. Woodward and E. C. Conrad, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 14.
6 W. Henning, *Deut. Lebensm.-Rundschau*, 79 (1982) 462.
7 K. Nagasawa, A. Ogamo and T. Shinozuka, *J. Chromatogr.*, 111 (1975) 51.
8 H. Small and T. E. Miller, *Anal. Chem.*, 54 (1982) 462.
9 M. Denkert, L. Hackzell, G. Schill and E. Sjögren, *J. Chromatogr.*, 218 (1981) 31.
10 M. Dreux, M. Lafosse and M. Pequignot, *Chromatographia*, 15 (1982) 653.
11 R. N. Reeve, *J. Chromatogr.*, 177 (1979) 393.
12 C. T. Hung and R. B. Taylor, *J. Chromatogr.*, 202 (1980) 333.
13 T. Korbelak, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 487.