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Spectrophotometric Determination of Dulcin (*p*-Ethoxy Phenyl Urea) in Foods

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ABSTRACT

A new spectrophotometric method for the determination of dulcin in foods has been developed. Dulcin is hydrolysed with 6N sodium hydroxide under reflux, the hydrolysed dulcin is oxidised at pH 3·0 with sodium hypochlorite and coupled with phenol at pH 10·0 to give an indophenol which absorbs at 630 nm. Beer's law is obeyed in a concentration range of 0·5–8·0 μg/ml with a percentage recovery range from 99·5–100.

INTRODUCTION

Dulcin (*p*-ethoxy phenyl urea), a non-nutritive sweetener prohibited under the provisions of The Prevention of Food Adulteration Act (1954) is still being used in many food products such as beverages, ice-candy, ice-cream, etc. Methods proposed earlier for its determination involve a spectrophotometric technique, based on Jorrisson's reaction (Andrea *et al.*, 1948; Akagi *et al.*, 1957), Nessler's method (Hirst *et al.*, 1941), the *p*-dimethylamino benzaldehyde method (Kawai, 1969), determination of

nitro derivative (Stoya, 1970), an ultraviolet spectrophotometric technique (AOAC, 1975) and fluorimetric assay (Uchiyama *et al.*, 1975, 1977). These methods are found to be rather tedious and time consuming. A new method described in this paper is based on hydrolysis, oxidation and coupling to give an indophenol derivative.

MATERIALS AND METHOD

Spectral and absorbance measurements were made with a Perkin-Elmer Coleman 575 model double beam digital spectrophotometer with 1 cm matched silica cells. A Toshniwal digital pH meter was used for all pH measurements.

All the solutions were prepared in double glass distilled water using Analar grade chemicals. Phenol (6% BDH, Analar); sodium arsenite (4%, E. Merck); dulcin (1 mg/ml, Riedel, Germany) were prepared in double glass distilled water. Sodium hypochlorite (4%) was prepared and standardised by the arsenious oxide method (AOAC, 1975).

Boric acid–sodium hydroxide buffer solution (pH 10·0) was prepared as described by Lurie (1975).

Preparation of standard curve

An aliquot of dulcin (10 mg) was placed in a conical flask fitted with a standard joint, sodium hydroxide was added to maintain an overall normality of 6N with respect to alkali and the contents of the flask were refluxed for 60 min. After cooling the excess alkali was neutralised using a calculated quantity of hydrochloric acid and made up to a known volume. Aliquots of this solution ranging from 50 to 400 μg were taken, 1 ml of 4% sodium hypochlorite was added and the pH was brought to 3·0 using 0·1N hydrochloric acid. After 1 min the excess hypochlorite was removed by adding 2 ml of 4% sodium arsenite. One millilitre of 6% phenol was added and the volumes were made up to 50 ml each with borate buffer (pH 10·0). The volumetric flasks were kept in a boiling water bath for 5 min, ccoled and the absorbance was measured at 630 nm.

Method for food products

An appropriate quantity of the food sample was made alkaline with 5 ml of 10% sodium hydroxide and extracted with 4 × 30 ml portions of

diethyl ether. The combined ether extract was washed once with distilled water, taken into a conical flask fitted with a standard joint and the solvent was removed. The rest of the procedure followed was as described in the preceding section, starting from a sodium hydroxide solution. The dulcin content was computed from the standard curve.

RESULTS AND DISCUSSION

The new indophenol formed showed maximum absorbance at 630 nm (see Fig. 1). The optimum pH for the oxidation of the *p*-amino phenol formed during the hydrolysis and coupling with phenol were found to be 3·0 and 10·0, respectively. Under the set experimental conditions Beer's law was

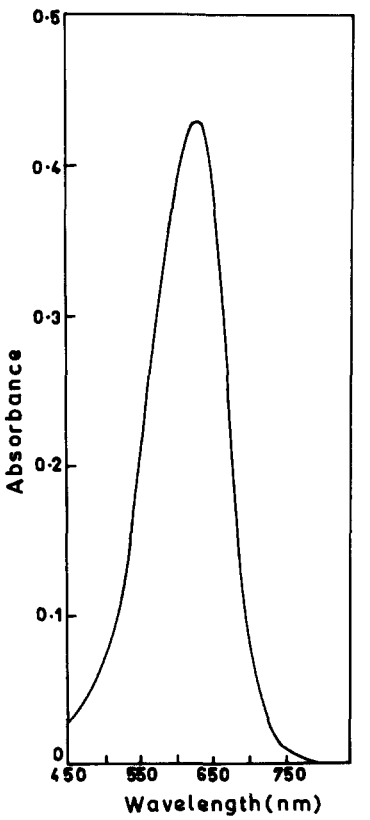

Fig. 1. Absorption spectrum of the indophenol formed from dulcin.

found to be obeyed over a concentration range of 0·50 to 8·0 μg/ml of dulcin. The molar absorptivity and the Sandal's sensitivity were found to be $2{\cdot}07 \times 10^4$ litres $mole^{-1}$ cm^{-1} and 0·0087 $\mu g\,cm^{-2}$, respectively. Comparison of the values of the recovery experiments of dulcin from various food products with that of the standard *p*-dimethyl amino benzaldehyde method (Kawai, 1969) revealed good recovery and accuracy (Table 1). The proposed method is better than reported colorimetric methods as it allows estimations in parts per million in the longer wavelength region where interference would naturally be less. By comparison of molar absorptivity values it could be seen that the proposed method was more sensitive than the methods reported earlier.

Food additives like benzoic acid, saccharin, salicylic acid and vanillin

TABLE 1
Recovery of Dulcin Added to Foods

Serial No.	*Name of the food product*	*Dulcin added (ppm)*	*Per cent recovery**	
			Proposed method	*p-dimethylamino benzaldehyde method*
1.	Control	50	99·5	98·8
2.	Control	200	99·8	98·9
3.	Control	1 000	100·0	99·7
4.	RTS beverage (Synthetic)	100	99·8	99·2
5.	RTS beverage (Mango)	100	99·6	99·0
6.	Ice-candy	200	99·6	100·0
7.	Ice-cream (Vanilla)	200	99·5	97·7

* Average of six individual determinations.

did not interfere in the estimation even if they were present in tenfold molar excess of dulcin. The effects of diverse anions like sulphate, chloride and phosphate on colour development were also studied and indicated no interference in the proposed method.

The coloured oxidative coupling product is found to be identical with that of *p*-amino phenol through spectral comparison. The proposed method is very simple and selective and can be used for the determination of dulcin with good accuracy in various food products.

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