Determination of Carbonyl Concentration in Aqueous Citrus Essences

A method for estimation of total carbonyl compounds in aqueous citrus essences as *n*-octanal is presented. This method is based on a determination of total carbonyl compounds as their dinitrophenylhydrazones colorimetrically and on an important modification in the preparation of the reagents. The range of carbonyl determination is 0 to 140 p.p.m. in the suggested method, as compared to 0 to 250 p.p.m. in the previously used method. A big advantage of the suggested method is that the absorption of an identical concentration of various aliphatic aldehydes, generally recognized to be in the essences, does not differ very much.

Attaway *et al.* (1967) suggested several methods for the determination of oxygenated terpene, aldehyde, and ester concentrations in aqueous citrus essences. Trying to use these methods for the evaluation of aqueous orange essences, we experienced difficulties with the Saturated Aliphatic Aldehyde method (SAA). This method is based on the ability of the aldehydes to catalyze the oxidation of *p*-phenylenediamine by hydrogen peroxide to produce a black product known as Bondrowski's base. The reaction was extremely time-sensitive and end-point determinations were hard to obtain. To overcome these difficulties, we adapted and modified a method, previously used by Critchfield (1963), for the determination of carbonyl compounds in aqueous citrus essences.

EXPERIMENTAL

Reagents. CARBONYL-FREE METHANOL. Five hundred milliliters of reagent grade methanol were refluxed for 2 hours with 5 grams of Girard P reagent and a few drops of concentrate HCl. After distillation through a short Vigreux column, the methanol was kept in tightly stoppered glass bottles.

DINITROPHENYLHYDRAZINE (DNPH) SOLUTION. Fifty milligrams of reagent grade 2,4-dinitrophenylhydrazine were dissolved in 25 ml. of carbonyl free methanol, 2 ml. of reagent grade concentrate HCl were added, and then diluted to 50 ml. with distilled water and kept in stoppered bottles at 5° C.

OCTANAL STOCK SOLUTION FOR CALIBRATION CURVE. One-half gram of reagent grade *n*-octanal was dissolved in 500 ml. of carbonyl free methanol (1000 p.p.m.).

Procedure. Pipet into a test tube 1 ml. of the sample and then successively add 1 ml. of the DNPH solution and 5 ml. of carbonyl free methanol. Allow to react 20 minutes at room temperature. Then add 10 ml. of pyridine stabilizer (80% pyridine in water) and 1 ml. of 33% KOH (in carbonyl free methanol) solution, and again allow to stand for 10 minutes at room temperature. Absorbance was read in a Bausch & Lomb Spectronic 20 colorimeter at 480 mμ.

RESULTS AND DISCUSSION

Results obtained by the original method (Critchfield, 1963) using double-distilled methanol, after 2 hours' reflux with 2,4-dinitrophenylhydrazine, indicated that calibration curves for *n*-octanal were not reproducible, and depended very much upon the reflux operation and the separation in the Vigreux column. However, the use of methanol treated in the same procedure with Girard P reagent gave satisfactory results after only one distillation, and much better results after a double distillation. All readings, using the Girard P treated methanol were higher, but curves were in all cases linear.

The time required for reaction between *n*-octanal and the DNPH solution was determined in a series of experiments. Another series of similar experiments was performed with aqueous citrus essences, obtained from a pilot-plant aroma recovery unit. Results indicated that 20 minutes were sufficient for the reaction between *n*-octanal and the DNPH, while 15 minutes were sufficient in the case of aqueous citrus essences. In the latter case, if reaction time is 20 minutes, the final readings would be about 1% lower. This difference in results was not considered to be important in our determinations, as it might be assumed that reaction rate would vary to some extent for the individual different carbonyls which contributed to this over-all value when compared to a single aldehyde.

To compare the DNPH method with the SAA method, for the determination of *n*-octanal, we used a standard solution of *n*-octanal and obtained straight line calibration curves. The calibration data (Table I) show that the DNPH method was much more reliable and easier to reproduce than the SAA method. The SAA method was previously reported (Attaway *et al.*, 1967) to be very sensitive to conditions of reaction such as time and temperature. Our findings reaffirmed these difficulties and also emphasized a dependence in the quality of the reagent grade H_2O_2, supplied to our laboratory from two different suppliers named source A and source B (Table I). These findings were the main reason for development of the DNPH method.

Determinations of carbonyls in citrus aroma aqueous solutions obtained in a pilot plant aroma recovery unit indicated the same trend in results as with calibration data (Table II). These results are representative data from similar analyses made with many different citrus essences. According to the DNPH method, aroma solutions 1 and 2 contain lower levels of carbonyl compounds than samples 3 and 4. However, based on the SAA method, samples 3 and 4 give lower optical density values than samples 1 and 2. This must be attributed to the difference in relative absorbance of different aldehyde solutions in the SAA method and the lower deviation in relative absorbance found in our experiment for several aldehydes in DNPH method (Table III).

Results (Table I) indicated that while the range of carbonyl determinations with SAA method was up to 250 p.p.m., it was up to only 140 p.p.m. using the DNPH method. The

Table I. Calibration Data for Determination of *n*-Octanal by SAA and DNPH Methods

DNPH Method			SAA Method					
			Using H_2O_2, Source A[a]			Using H_2O_2, Source B[a]		
n-Octanal Concentration (p.p.m.)	Absorbance (Av.)	Absorbance (Range of Readings)	*n*-Octanal Concentration (p.p.m.)	Absorbance (Av.)	Absorbance (Range of Readings)	*n*-Octanal Concentration (p.p.m.)	Absorbance (Av.)	Absorbance (Range of Readings)
20	0.09	0.090–0.090	100	0.28	0.24–0.31	40	0.12	0.10–0.14
40	0.19	0.185–0.195	200	0.56	0.50–0.64	75	0.37	0.31–0.31
80	0.38	0.375–0.485	300	0.83	0.75–0.90	120	0.92	0.40–0.44
100	0.46	0.45–0.47	400	1.13	0.94–1.3	150	0.56	0.52–0.61
140	0.66	0.66–0.67				250	0.73	0.68–0.76
200	0.92	0.92–0.94				350	1.25	1.20–1.40

[a] At least 8 determinations were perfomed

Table II. Determination of Carbonyl Content of Aroma Solutions by Two Methods

		DNPH Method, Absorbance at 480 mμ		SAA Method, Absorbance at 435 mμ	
Sample	Dilution	Av.[a]	Range of Readings	Av.[a]	Range of Readings
1	1:5	0.30	0.30	0.52	0.50–0.54
2	1:5	0.245	0.240–0.250	0.50	0.50
3	1:10	0.56	0.56	0.20	0.19–0.21
4	1:10	0.55	0.55	0.16	0.16–0.20

[a] Av. of at least 4 determinations.

Table III. Absorbance of Aldehydes in Carbonyl-Free Methanol Determined by DNPH Method

Aldehyde	Concentration, p.p.m.	Absorbance at 480 mμ
n-Propionaldehyde	100	0.47
n-Butyraldehyde	100	0.60
n-Octanal	100	0.45
n-Decanal	100	0.28

DNPH calibration curve remained linear up to 200 p.p.m. but since absorbance values at the latter concentration were 0.7, it was decided not to use this range. The difference in ranges can easily be overcome by diluting samples with carbonyl-free methanol or distilled water without affecting linearity of results. The minimum detectable level of *n*-octanal was 2 p.p.m. by our instrument using a 10-mm. test tube. The detection level can be increased using a $^3/_4$-inch test tube.

The spectrum of *n*-octanal-2,4-dinitrophenylhydrazone in the test medium obtained in a Beckman DB spectrophotometer operating at 40 mμ per minute is given in Figure 1. The spectrum is similar to the generally recognized dinitrophenylhydrazones having a peak around 430–440 mμ and a plateau from 480 to 520 mμ.

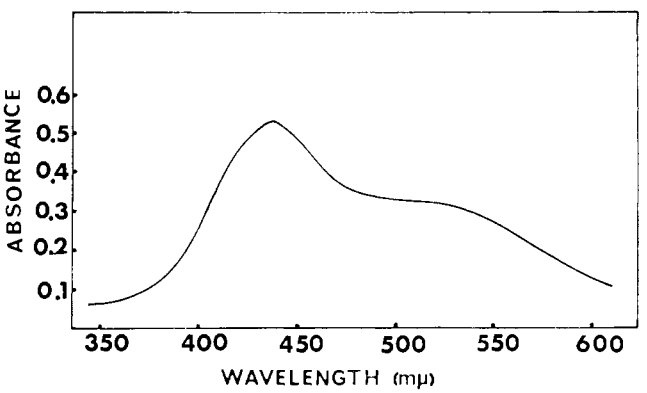

Figure 1. Absorption spectrum of *n*-octanal-2,4-dinitrophenylhydrazone

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