may be used successfully in the spectrophotometric dosage of nitrazepam, also for the rapidity and facility of working procedures.

Experimental. Visible spectra were recorded using a Rank Precision, Uvichem H 1600 S.T. Spectrophotometer; 1-cm stoppered fused silica cells were used. Nitrazepam (F.I.S. SpA, Vicenza, Italy) and 1,2-naphtoquinone (Fluka AG, Buchs, Switzerland) were purified by crystallization from ethanol and benzene respectively. Dimethylformamide was reagent grade for spectrophotometry. Solutions of nitrazepam and of 1,2-naphtoquinone in DMF, both of concentration 5×10^{-4} *M* were prepared. The DMF solution of nitrazepam contains also the 10% by volume of ammonium hydroxide (26° Bé). Solutions of nitrazepam and of 1,2-naphtoquinone were mixed in molar ratio 1:1, 1:2 and 1:4, and the optical densities of these mixtures were measured: the absorption spectra (Figure 1) show that the curves do not cross in the explored region of the spectrum. Then, a series of mixtures was prepared by adding x ml of 5×10^{-4} *M* 1,2-naphtoquinone solution to $(1-x)$ ml of 5×10^{-4} *M* nitrazepam solution,

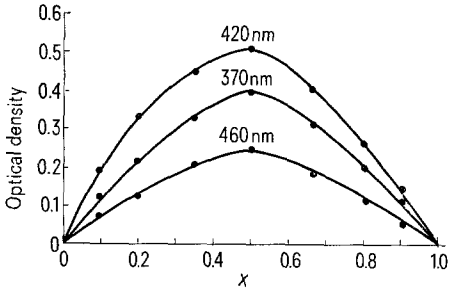

Fig. 2. Absorption spectra of mixtures of x ml of 5×10^{-4} *M* 1,2-naphtoquinone solution with $(1-x)$ ml of 5×10^{-4} *M* nitrazepam solution.

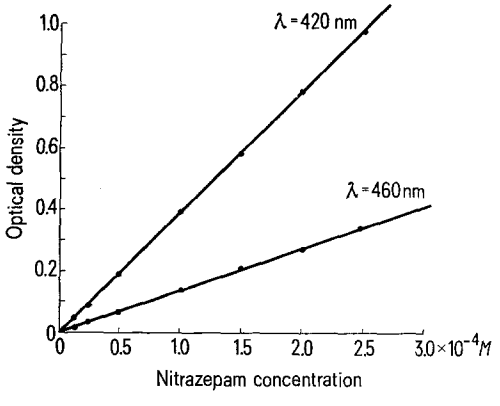

Fig. 3. Plots of optical density vs. nitrazepam concentration according to the law of Lambert-Beer.

and the optical densities of these mixtures were measured at wave lengths of 370, 420 and 460 nm (Figure 2). All wave lengths gave a definite maximum at $x = 0.5$: this is what would be expected for substances that react only in the molar ratio of one to one. In order to establish the range of nitrazepam concentration in which the optical density of the DMF solution containing the 2 components follows the law of Lambert-Beer, a series of samples was prepared by adding known and increased volumes (in the range 0.25 ÷ 5 ml) of ammoniacal DMF solution of nitrazepam to 5 ml of DMF solution of 1,2-naphtoquinone; the samples were diluted to constant volume of 10 ml with DMF and allowed to stand for 5 min before reading the optical densities. The reference sample was made by adding 1 ml of ammonium hydroxide (26° Bé) to 5 ml of DMF 5×10^{-4} *M* 1,2-naphtoquinone solution, and diluting to 10 ml. Figure 3 shows the linear correlation between optical density and nitrazepam concentration in the range $0.125 \times 10^{-4} \div 2.5 \times 10^{-4}$ moles/l, corresponding to a content of nitrazepam between 3.5 and 70 µg/ml. The reliability of this colorimetric determination in toxicological chemistry was verified on samples of blood, saliva and urine. To this purpose, one-ml samples of biological liquid of known and variable amounts (in the above range) of nitrazepam (DMF solution 5×10^{-4} *M*) were added. Each one of these samples was treated with 2 ml of 30% aqueous solution of trichloroacetic acid; after stirring for a few min, the precipitate was filtered and then washed with 1 additional ml of trichloroacetic acid. The collected filtrate was concentrated (without loss) by moderate heating and, after cooling, mixed with 1 ml of ammonium hydroxide (26° Bé) and 1 ml of 2.5×10^{-3} *M* 1,2-naphtoquinone DMF solution. The volume was made up to 10 ml and after 5 min the extinction of the colored solution was measured at 420 nm. The reference sample was the same as mentioned above. The quantity of nitrazepam evaluated in the blood-serum was 95 ± 1.5% of the added amount; in saliva 96 ± 2% and in urine 94 ± 3%. All the manipulations require a time of 20 min.

Riassunto. È stata studiata per via spettrofotometrica la reazione cromatica tra nitrazepam e 1,2-naftochinone in dimetilformammide (DMF) in presenza di ammoniaca. È stato stabilito che in soluzione si forma un addotto molecolare dei due reagenti in rapporto molare 1:1. Tale reazione viene proposta come metodo di determinazione colorimetrica del nitrazepam in liquidi biologici quali sangue, saliva ed urina.

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