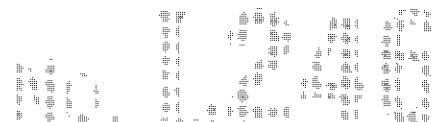


HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



Application of the New Photodiode Array UV-VIS Detector SPD-M10A(No.2)

The photodiode array detector has come to be used as a detector for HPLC

in various fields like analysis of medical and pharmaceutical analyses and food analyses, the photodiode array detector, which allows acquisition of not only chromatogram information but spectral information, has become indispensable for the confirmation of target analytes and for the evaluation

of foreign substances. In accordance with the spread of the concept concerned with GLP/GMP, it is expected that the photodiode array detector will become a more and more important detector. Introduced in this article are analyses of imazalil which is used as fungicide for citrus fruits and analysis of impurities of gabexate mesilate accommodated in the Japan Pharmacopoeia, revision No.13. (J.Masuda)

■ Analysis of Imazalil

Imazalil is used as fungicide for oranges and bananae imported. Here, analysis of imazalil contained in imported oranges is introduced. The sample was pretreated in accordance with the method indicated by the Hygienics Test Standard (Appendix 1995). In actual analysis, it is necessary to confirm the target substance from among many foreign substances. For that purpose, the function of the photodiode array detector on the spectrum and peak purity of the target substance displays its power. The analysis introduced here enables comparison of the spectra of the peaks of the standard sample and the actual sample corresponding to imazalil, ensuring higher reliability in quantitation and determination. As the results of analysis, 0.0018g/kg of imazalil was detected in an imported orange.

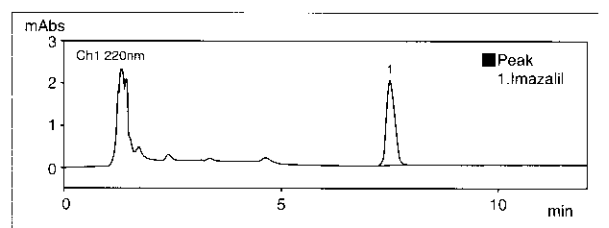


Fig.1 Chromatogram of standard solution of Imazalil

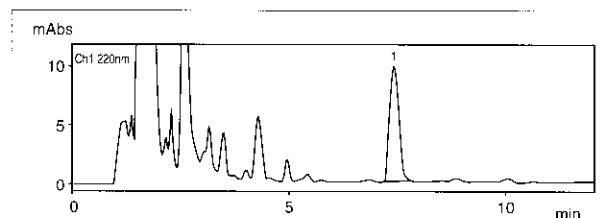


Fig.2 Chromatogram of Imazalil Imported Orange(220nm)

Table 1 Analytical Conditions

Colum	: STR ODS-II (4.6mmI.D.×150mmL.)
Mobile Phase	: 10mM (sodium) phosphate buffer (pH=6.9/ Acetonitrile=45/55
Flow Rate	: 1.0mL/min
Temperature	: 40°C
Detection	: SPD-M10Avp (210nm~300nm)
Injection Volume	: 10 µL

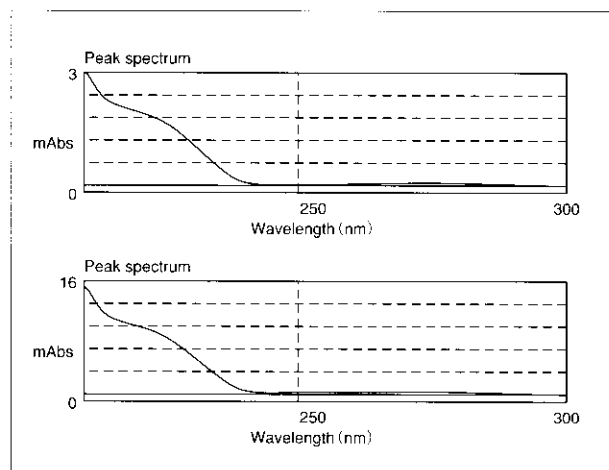


Fig.3 Spectra of Imazalil in Standard Solution (upper tier) and in a Sample (Lower tier)

■ Analysis of Gabexate Mesilate

According to the guideline indicated by the Ministry of Health, it is necessary to report the chemical structure and features of an impurity contained in a drug substance at or above an apparent level of 0.1% or an impurity of which strong toxicity or strong

pharmacologic effect is anticipated, even if its concentration may be less than 0.1%. In relation to this, it is considered that 0.1% of the drug substance is a rough criterion when performing analysis of impurities.

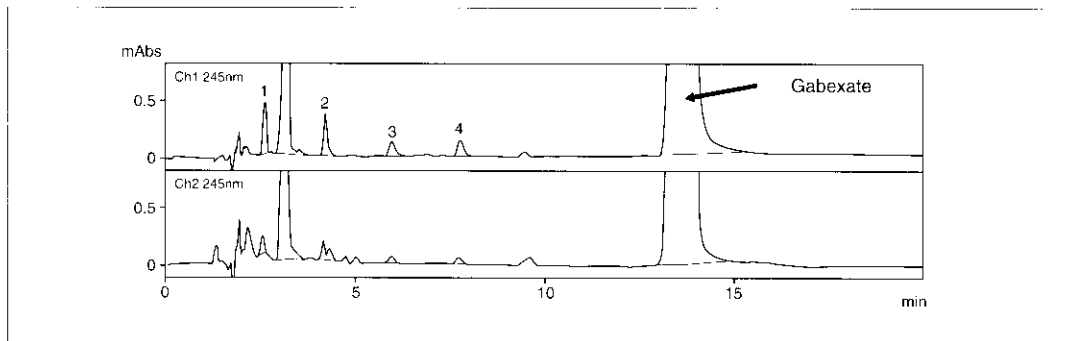


Fig. 4 Chromatograms of Gabexate (245nm, 250nm)(250 µg/mL as Gabexate Mesilate)

Shown in Fig.4 is a chromatogram of Gabexate Mesilate. Further, Fig.5 shows spectra of peaks that can be found at 245nm and are considered to be related to the drug substance. From these, it is comprehended that even in case of a low concentration impurity, spectrum can be obtained with sufficiently high sensitivity. Furthermore, with respect to Peak No.2, from the peak purity curve shown in Fig.6 and by comparing chromatograms at 245nm and 220nm, overlapping by foreign substance can be clearly confirmed. For such an analysis, information of multi-wavelength and high-sensitivity is very effective.

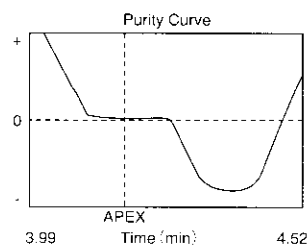


Fig.6 Peak Purity Curve of Peak No.2

Table 2 Analytical Conditions

Column	: STR ODS-II (4.6mmI.D. × 150mmL.)
Mobile Phase	: 10mM (sodium) phosphate buffer (pH=6.9/ Acetonitrile=45/55
Flow Rate	: 1.0mL/min
Temperature	: 40 C
Detection	: SPD-M10Avp (210nm~300nm)
Injection Volume	: 5 µL

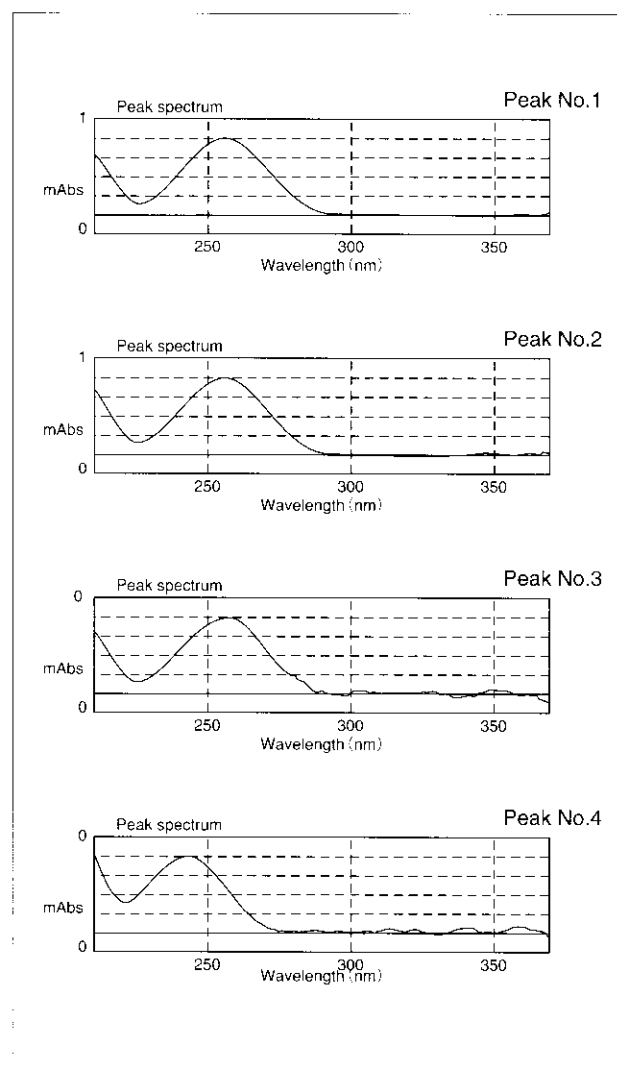


Fig. 5 Spectra of Peaks of Related Compounds in Chromatogram (245nm)

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