Table 2. Per cent identity of the coat protein and 3′ non coding region of TMV (Tom-K) with other tobamoviruses

| Virus | Nucleotide sequence of CP | Nucleotide sequence of 3′ NC | Deduced amino acid sequence of CP | Ref. |
|---|---|---|---|---|
| Tobacco mosaic virus strain vulgare (TMV) | 92.6 | 97.6 | 94.3 | 20 |
| Tomato mosaic virus (ToMV) | 72.7 | 77.3 | 78.6 | 21 |
| Odontoglossum ring spot virus (ORSV) | 65.8 | 69.5 | 70.5 | 22 |
| Pepper mild mottle virus (PMMV) | 64.5 | 63.3 | 68.2 | 23 |
| Tobacco mild green mosaic virus (TMGMV) | 62.9 | 35.7 | 66.7 | 24 |
| Cucumber green mottle mosaic virus (CGMMV) | 45.7 | 56.0 | 35.8 | 25 |

strain of TMV and not a strain of ToMV. This is further supported by the data presented in Table 2 which depicts the per cent identity of the coat protein and 3′ non-coding region of the present isolate with other tobamoviruses. Figure 3 shows the dendrogram of the tobamoviral CP sequences. Even here TMV (Tom-K) clusters with TMV CP rather than with ToMV CP. Comparison of the CP sequence of TMV (Tom-K) with the TMV strain vulgare[20] showed that there are 9 amino acid changes between TMV CP and that of the Indian isolate (Figure 4). Out of these, 5 of the changes are conservative substitutions. On the other hand, 32 amino acid changes were observed between TMV (Tom-K) and ToMV[21]. Thus the analysis of the 3′ terminal nucleotides of the Indian isolate clearly establishes that the isolate is a strain of tobacco mosaic and hence is called tobacco mosaic virus tomato strain (TMV (Tom-K)).

The nucleotide sequence reported in this paper has been submitted to GenBank and assigned the accession number AF 126505.

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# Serotyping of foot-and-mouth disease virus from aerosols in the infected area

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**A simple and reliable test was standardized for serotyping foot-and-mouth disease virus from aerosols. The test, which is based on antigen capture-RT/PCR method, detected the serotype of the virus in the air exhaled by a diseased bull. The test has also been successfully used to detect the virus serotype in the aerosols from the containment area where the tissue culture virus was handled. Antibodies raised against recombinant proteins of all the serotype were used in the test to improve the specificity.**

FOOT-and-mouth disease (FMD) is one of the most important diseases of livestock such as cattle, sheep, goat

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and pigs. The causative agent, foot-and-mouth disease virus (FMDV) belongs to genus *Aphthovirus* of the family *Picornaviridae*[1]. The virus particle which sediments at 146S consists of a single stranded positive sense RNA molecule of about 8.5 kb (ref. 2) with a molecular weight of $2.6 \times 10^6$ daltons enclosed in a capsid which is composed of 60 copies each of four structural proteins named VP1, VP2, VP3 and VP4. VP1 is exposed on the surface of the virion and has immunogenic property[3]. India has remained an ideal habitat for the infection to persist, spread and flourish over centuries with its agro-climatic and socio-economic conditions conducive to the perpetuation of the virus. It has mixed farming of different species of livestock, which altogether provides a favourable ecological milieu for the pathogen. Epidemiology of FMD is complex and rather difficult to understand with its changing pattern of frequency, multiplicity of types and variants, ease of transmission and spread and the total number of disease outbreaks that occur over a particular period of time. The disease is endemic to India with four (A, O, C, Asia-1) serotypes in circulation besides numerous field variants. In countries like India, where slaughtering policy cannot be adopted due to various reasons, the disease can only be controlled by regular vaccination supported by early diagnosis, proper disposal of infected animals and restricted animal movement. Since inactivated viral vaccines against FMDV confer short duration of immunity and continued disease outbreaks and variations among circulating viruses are the major problems of control programmes, it is essential to have rapid and sensitive diagnostic tests. Various serological, immunological, and biochemical tests have been developed for specific diagnosis of FMD in field samples. Of late, sensitive tests based on PCR and nucleic acid hybridization have been developed[4–7]. Most of these are related to the major immunogen VP1 as type-specific amino acid sequence variations have been found at the carboxy terminal end of this protein. The homology in this region with respect to the nucleic acid sequence is less than 60% among the various types[6,8,9]. Probes corresponding to this region have been used for FMDV typing[10] and sequence information was used to study the strain variation[11,12]. Application of nucleic acid probes is time consuming and needs trained manpower and cost intensive infrastructure. However PCR based methods are highly sensitive, less time consuming and can be adopted for routine use. The PCR method was modified and used for virus typing. The modified technique termed antigen capture RT/PCR (Ag-RT/PCR) was found to be 100 times more sensitive than ELISA[13]. These tests were conducted routinely on saliva or tongue epithelia collected from the diseased animal. However, collection of these samples for diagnostic purpose is associated with physical handling of the animal and thereby increasing the risk of spreading the virus to the environment, which is not desirable. In order to avoid this risk, we have a standardized simple method, which uses the aerosol samples for virus type detection by Ag-RT/PCR. The test not only detected the virus type from the aerosol near the affected animal, but also from the area where the virus is handled.

BHK 21 clone 13 passaged FMDV maintained as vaccine virus at the Indian Veterinary Research Institute (IVRI), Bangalore was used as positive control. Ten per cent tongue epithelial extract was prepared in PBS (8 g of NaCl, 0.2 g of KCl, 1.44 g of $Na_2HPO_4$ and 0.24 g of $KH_2PO_4$, pH 7.4) and used. Air collected near the animals during the recent outbreak of the disease in Bangalore or from the containment area where the cell culture virus was handled, was used as test sample. In the first case, 50 ml of air was drawn from the vicinity (12 cm away) of the affected animals using a sterile plastic syringe without touching the animal, feed or water and bubbled slowly into 200 µl of sterile distilled water in 1.5 ml microcentrifuge tube. Ten µl of the sample was used for capturing and detection. In the second case, 50 ml air was drawn near the working place (10 feet away from the actual handling spot) and passed into 200 µl of sterile distilled water and 10 µl of the sample was subjected as per the procedure described by Suryanarayana *et al.*[7], except that antibodies against *E. coli* expressed truncated proteins were used in the test in place of antibodies raised against the whole virus.

Application of Ag-RT/PCR test for virus serotyping was reported[13]. Briefly, a set of microcentrifuge tubes coated with all the four type-specific antibodies (against immuno-reactive recombinant proteins) was used for virus capturing. Ten µl of the virus solution (either from the aerosol or tissue extracts) was mixed with 100 µl of PBS-Tween 3% BSA and subjected to antigen capture in antibody-coated tubes for 2 h at 37°C. The bound antigen in the tubes were subjected to RT/PCR as described elsewhere[13]. In order to study the use of this test on aerosols for virus typing, the air collected from the containment area before and after handling of FMDV serotype Asia I and A22 (with one day interval in between) was subjected to the assay. There was no amplification of the 330 bp DNA in the air collected before handling of the virus (Figure 1, lanes 2 to 5). Intense DNA bands corresponding to Asia I and A22 were seen in the lanes 9 and 11, respectively. No non-specific amplification of DNA in other heterologous antibody-coated tubes (lanes 6 to 8 and 12 to 14, respectively) were found. When the test was further extended to identify the presence of the virus in the containment area one day after handling of the Asia I virus, the air collected showed the amplified DNAs corresponding to Asia I virus (lane 18) indicating that the test is sensitive enough to detect even a minute quantity of virus in the aerosol. Encouraged with the sensitivity and specificity of the technique of detecting the virus in aerosol sam-

ples, we have tested the method on the air collected (in duplicate) from the vicinity of the diseased animals kept separately in animal sheds. Subsequently, the epithelial extracts of the affected animals were also tested for confirmation of the results from the aerosol (Figure 2). Intense amplified DNA corresponding to Asia I was seen in both the aerosol samples (lanes 5 and 9) and epithelial extract (lane 13) indicating that the virus causing the disease was FMDV serotype Asia I and the released virus particles in the aerosol could be trapped and the specific gene amplified. There was no non-specific amplification as observed by the absence of DNA bands in other lanes (lane 2 to 4, 6 to 8 and 10 to 12).

This test was repeated on several other aerosol samples, which include air samples from guinea pig cages housing infected guinea pigs and laminar floors. In case of FMD, which is highly contagious and spreads rapidly, handling of infected tissue for diagnostic purpose is undesirable. In countries like India where FMD is endemic and FMDV occurs as four serologically distinct serotypes, rapid diagnosis by way of serotyping is essential for successful control of the disease. Initially, FMD was diagnosed by conventional tests like CFT and virus neutralization. Later nucleic acid-based probes have been developed for virus detection in infected animals. With the advent of highly sensitive PCR methods, the detection of strain variation by sequencing was possible[14]. The Ag-RT/PCR developed by us was found to be highly sensitive for virus typing. Use of antibodies against recombinant proteins further added to the sensitivity and specificity of the test (manuscript under preparation).

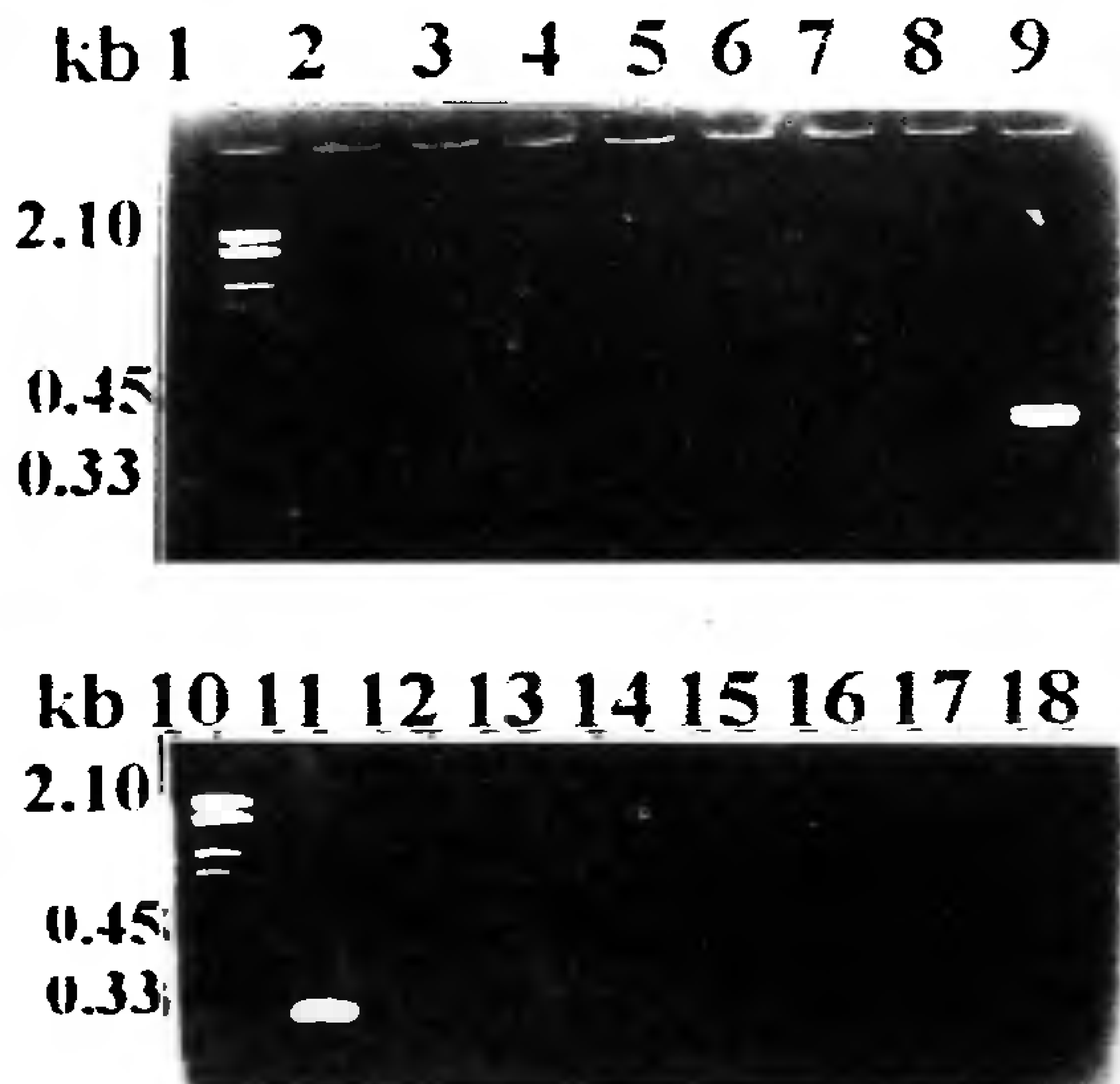


Figure 1. Agarose gel electrophoresis of PCR amplified DNA from aerosols. Air was collected, virus captured and subjected to Ag-RT/PCR. The products were analysed by 1.5% agarose gel electrophoresis. Amplified DNA from virus handling room, lane 2–5, before handling; lane 6–9, after handling FMDV type Asia I; lane 11–14, after handling A22 virus; and lanes 15–18, one day after handling Asia I virus. A (2, 6, 11, 15) or O ( 3, 7, 12, 16) or C (4, 8, 13, 17) or Asia I (5, 9, 14, 18) coated tubes. Lane 1 and 10, molecular weight marker.

Figure 2. Agarose gel electrophoresis of PCR amplified DNAs from aerosols and epithelium. Cattle tongue epithelial extract or aerosol sample collected from the infected area was subjected to Ag-RT/PCR and the amplified products were analysed by agarose gel electrophoresis. The amplified products are: lane 2 to 5, virus captured from cattle tongue epithelial extract; lanes 6 to 9 and 10 to 13, aerosol samples collected in A22 (2, 6, 10), O (3, 7, 11), C (4,8,12) and Asia I (5, 9, 13) antibody-coated tubes.

We have indicated the scope of this technique in the detection of the virus from contaminated water and aerosols in our earlier studies. This has been found true in the present report, which is the first of its kind. We feel that the test can be adapted regularly for typing the virus released out in the air without physically handling the animal for want of infected tissue. This simple method will avoid unnecessary spread of the virus in the environment. Our studies in the containment area showed that the test may be employed for checking the presence of the virus type in the P3 laboratories or in the air exhausts of the virus handling units or in any other contaminated area.


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# Identification of alpha-terthienyl radical *in vitro*: A new aspect in alpha-terthienyl phototoxicity

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**Alpha-terthienyl, the naturally occurring larvicide from tagetes species, exhibits unique free radical generating property. Besides generating singlet oxygen and superoxide anion radical, it generates alpha-terthienyl radical, when exposed to ultraviolet light. This free radical generating property of alpha-terthienyl can be utilized to understand the mechanism of action against insects; however this use also poses a threat to the non-target species exposed to it.**

LIGHT-activated pesticides are recognized as a new technology with considerable promise in the area of pest control. Erythrosin B has already been registered for housefly control, and a promising photodynamic herbicide based on porphyrin metabolism is under development[1]. Among the most active biocides whose activity is enhanced by light are the naturally occurring thiophenes and biosynthetically-related polyacetylenes which are characteristic secondary plant metabolites of the plant family Asteraceae[1]. For example, alpha-terthienyl ($\alpha$-T) (Figure 1) has been extensively studied and evaluated as a larvicide[2], fungicide[3] and nematocide[4]. Several attempts have been made to examine the phototoxicity of terthienyl and bithenyl analogs[5–8], which have been moderately successful in improving our understanding of the structure–activity relationship and for improving efficacy. $\alpha$-T gives an absorption peak at 351 nm in ethanol (Figure 2) and generates singlet oxygen[6] and superoxide anion radical both *in vivo*[9,10] and *in vitro*[10,11] under ultraviolet (UV) light.

A stock solution of 2 μg/ml of $\alpha$-T was prepared in absolute ethyl alcohol. Ten μl of $\alpha$-T solution was added to 100 μl on *n-t*-butyl-*a* phenyl nitrone (PBN) (50 mM, final concentration). The total mixture of 100 μl was irradiated with ultraviolet light (320–400 nm) for different time intervals from 1 to 120 s (1, 2, 5, 10, 15, 30, 45, 60, 90 and 120 s). Another tube containing the same reagents was kept in total darkness for 1 h, which served as control. After incubation, 50 μl aliquots from each of the above samples were transferred to glass capillary tubes and one end of each capillary tube was flame-sealed.

Electron paramagnetic resonance (EPR) spectra of the PBN free radical adducts formed were then recorded on a Varian E-104 EPR spectrometer (Pal Alto, USA) equipped with a $TM_{110}$ cavity, and were compared with the reference spectrum of superoxide radical generated by autoxidation of pyrogallol[12], which served as a control. Instrument settings were as follows: field set, 3237 G; temperature, 27 + 5°C; scan range, 10 × 10 G; time constant, 0.5 s; scan time, 4 min; microwave power, 5 mW; microwave frequency, 100 kHz; modulation amplitude, 2 G; modulation frequency, 9.01 GHz; and receiver gain, 1.25 × 104 × 10 (unless otherwise stated).

Figure 3 shows spin-trapping results of free radicals formed by exposure of $\alpha$-T to UV light. The EPR signals obtained by ultraviolet irradiation of $\alpha$-T for 1, 2, 5, 10, 15, 30 and 40 s (Figure 3 *a–g*) exhibited an EPR signal with three lines. The line intensities were 1:2:1 (aN = 16 G) which differed from the line intensities of

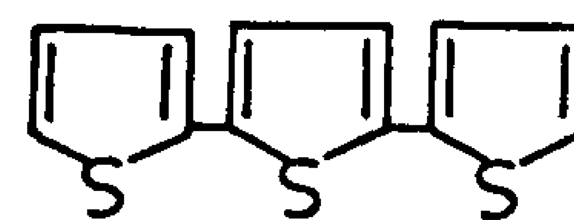


**Figure 1.** Chemical structure of alpha-terthienyl.

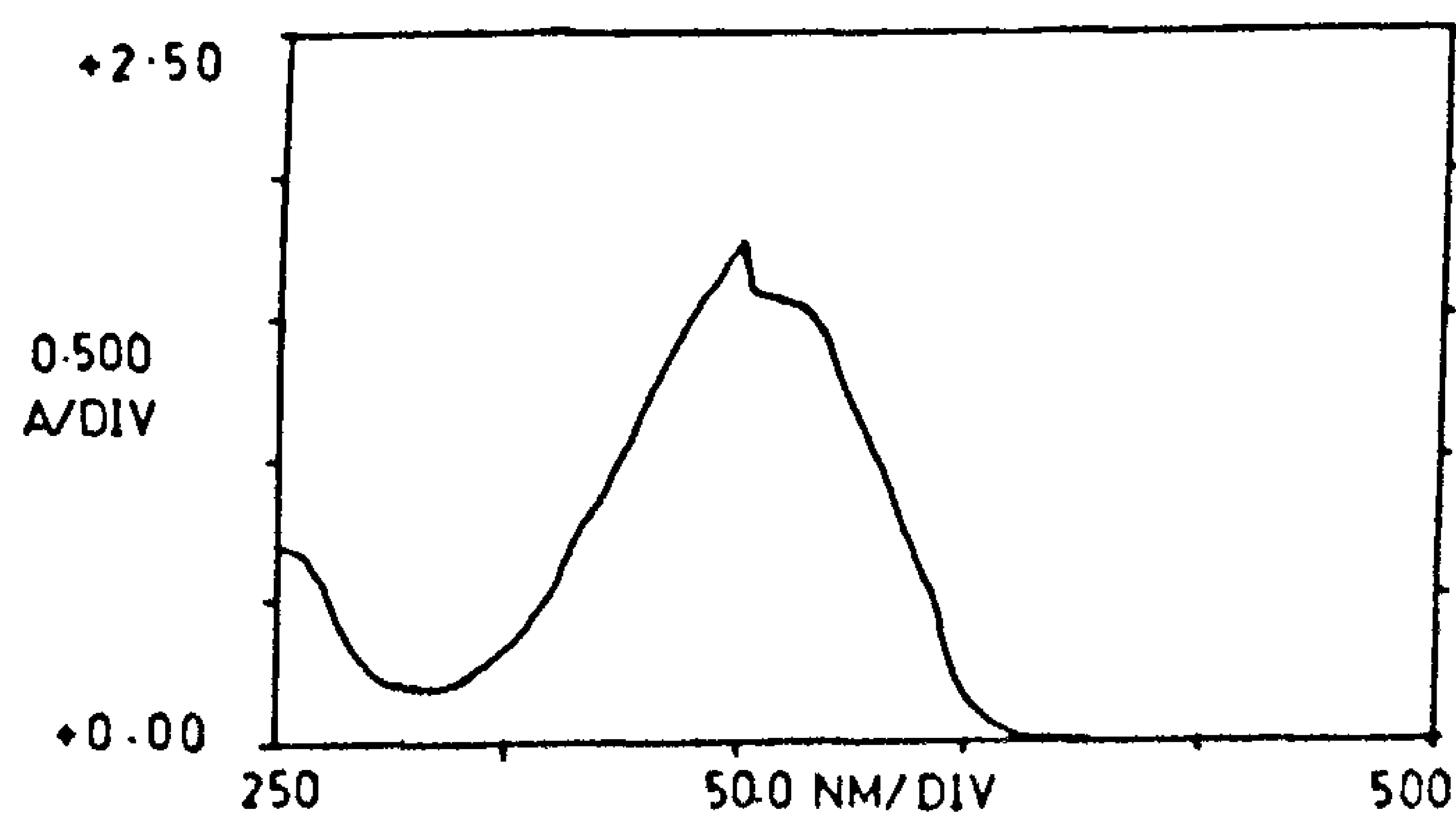


**Figure 2.** UV-VIS absorption spectrum of alpha-terthienyl showing a sharp absorption peak at 351 nm.